992- September 30, 1993

93 annual report

Division Of

Cancer Prevention and Control

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

NATIONAL CANCER INSTITUTE

Intramural Activities

October 1, 1992- September 30, 1993

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DIRECTOR'S REPORT

This report describes the intramural research activities of the Division of Cancer Prevention and Control (DCPC), one of the five program divisions of the National Cancer Institute (NCI). The mission of the DCPC encompasses basic and applied research on cancer prevention, cancer control research, public health applications research including technology transfer, and cancer surveillance, all aimed at the overall goal of the NCI: to reduce the incidence, mortality, and morbidity of cancer. Intramural research is one of the foundation stones of both the National Cancer Institute and the Cancer Prevention and Control Program.

The DCPC conducts the full spectrum of research on prevention and control, from the earliest stages of hypothesis development through clinical studies and trials, through defined population studies, all leading to a program of demonstration and evaluation studies. The Division's activities include research on prevention, evaluation of screening and early detection regimens, research on cancer among special populations, and research on rehabilitation and continuing care. A major emphasis is cancer prevention research, with significant efforts (particularly in the intramural program) devoted to research on diet, nutrition, and chemoprevention. As outlined below, the DCPC's intramural program is composed of three Branches and one Laboratory.

ORGANIZATION

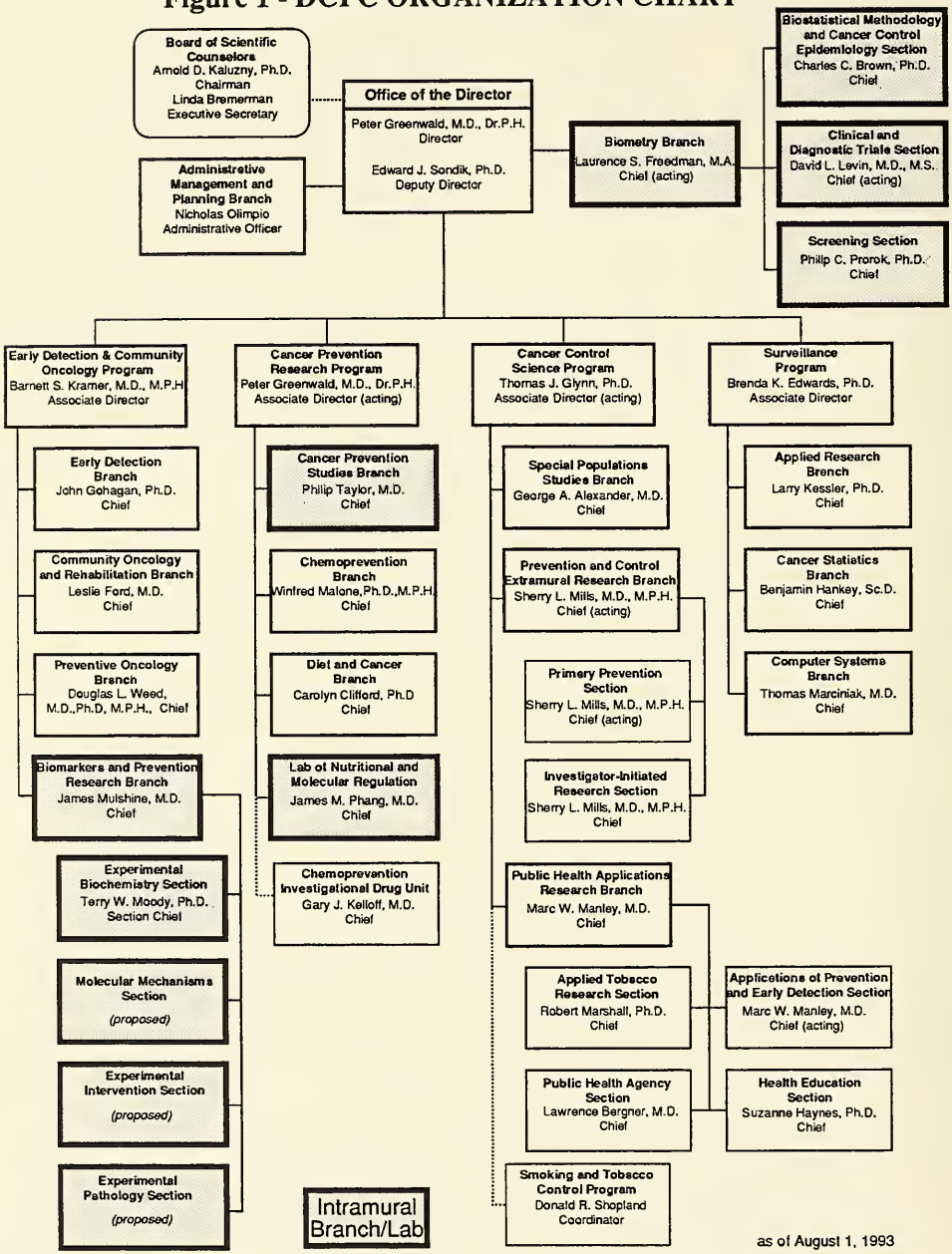
Figure 1 outlines the DCPC organization. The Division consists of four major programs, each led by an Associate Director. The Office of the Division Director provides overall coordination and direction as well as analytic program support. Each program is described briefly below.

The **Office of the Director** is responsible for the coordination and direction of the Division programs. It includes two branches: the Biometry Branch (one of the intramural components) and the Administrative Management and Planning Branch. The Biometry Branch conducts and supports intramural research using epidemiologic databases, intramural research in biostatistics methodology and aspects of cancer screening, and clinical trials research. The Administrative Management and Planning Branch assists in the management of the Division's budget and administrative matters.

The **Cancer Prevention Research Program** (CPRP) is charged with planning and supporting both intramural and extramural research in diet, nutrition and cancer, and chemoprevention. In addition, this organizational unit serves as the focal point for coordinating diet, nutrition, and cancer activities across the NCI divisions. This Program houses the Cancer Prevention Studies Branch (CPSB) and the Laboratory of Nutritional and Molecular Regulation (LNMR), two of the four intramural components of the Division. The Laboratory is located at the NCI-Frederick Cancer Research and Development Center (NCI-FCRDC) in Frederick, Maryland. The extramural units of the Program are the Chemoprevention Branch, responsible for large-scale trials and investigator-initiated trials on chemoprevention regimens; the Diet and Cancer Branch, which focuses investigator-initiated research on diet and nutrition, including the development of diet-based clinical trials; and the Chemoprevention Investigational Drug Unit, which systematically screens and evaluates potential chemopreventive agents and guides their development through the clinical research phases.

The **Early Detection and Community Oncology Program** (EDCOP) supports the community-based clinical research programs, as well as early detection and rehabilitation research. The Program includes the fourth of the Division's intramural components, the Biomarkers and Prevention Research Branch (BPRB). The Branch focuses on the use of molecular biological techniques and clinical medicine to develop the biomarker tools to prevent and find cancer early. These programs are designed to improve the delivery and application of state-of-the-art cancer regimens.

Figure 1 - DCPC ORGANIZATION CHART



The EDCOP extramural units include the Early Detection Branch, which supports research aimed at reducing cancer morbidity and mortality through appropriate early treatment; the Community Oncology and Rehabilitation Branch, which coordinates the Community Clinical Oncology Program (CCOP) and the minority-based Community Clinical Oncology Program—these CCOPs link community-based physicians with Cancer Centers and the Cooperative Groups to conduct clinical treatment research and cancer prevention and control research in community settings; and the Preventive Oncology Branch, which conducts the Division's Cancer Prevention Fellowship Program, providing an opportunity for physicians and scientists to train and gain field experience in cancer prevention and control by working with DCPC preceptors.

The **Cancer Control Science Program** (CCSP) supports research on ways to effectively transfer cancer control information to the public and to physicians, nurses, and other health professionals. This Program's efforts are directed toward study of a wide variety of cancer control intervention strategies to assess both their impact on populations and the use of proven cancer control methods. Programs that involve State, local and volunteer health groups, and populations that suffer disproportionately from cancer figure prominently in the Program's activities. The Program also directs a number of cancer control resource activities, including the National Black Leadership Initiative on Cancer. The Program is organized into three extramural units: the Special Populations Studies Branch, the Public Health Applications Research Branch, and the Prevention and Control Extramural Research Branch. The Smoking and Tobacco Control Program, which provides liaison between NCI, NIH, and the Department of Health and Human Services, is another important component of the CCSP.

The **Surveillance Program** is responsible for tracking and evaluating trends in cancer and for research on quantitative methods and statistics designed to monitor progress in cancer control for the United States. An important part of the Surveillance Program is a network of population-based cancer reporting systems the Surveillance, Epidemiology, and End Results (SEER) Program. Related efforts gather and disseminate information on cancer, cancer risk factors, and other elements of cancer control through a variety of reports. The Program also conducts studies on the organization, delivery, and financing of cancer prevention and control services, as well as on the economics of cancer. The Surveillance Program includes three branches: the Cancer Statistics Branch, responsible for the SEER program; the Applied Research Branch, which conducts a variety of analytic and methodological studies and develops methods related to cancer surveillance and the evaluation of cancer control; and the Computer Systems Branch, which provides comprehensive computer systems analysis, design, operation, and programming support for the Division.

INTRAMURAL ACTIVITIES

The overall goal of the Intramural Program is to conduct research that defines the state of the science for cancer prevention and control. In addition, the Program conducts methods research fundamental to the development of technical approaches underlying many large-scale projects in cancer prevention and control research. Within the DCPC, intramural research is conducted through the Biometry Branch (BB), the Cancer Prevention Studies Branch (CPSB), the Laboratory of Nutritional and Molecular Regulation (LNMR), and the Biomarkers and Prevention Research Branch (BPRB).

The **Biometry Branch** plans epidemiologic methodology and investigates mathematical modeling of processes relevant to cancer prevention and control activities. The Biometry Branch also provides consultation on statistical methodology and study design within the Division and to other scientists both within and outside the NIH.

The **Cancer Prevention Studies Branch** (CPSB) conducts a variety of prevention research but focuses on controlled intervention studies. Intervention studies serve the dual purposes of confirming hypotheses about cancer etiology and effecting cancer control, and act as a bridge between these two types of research efforts. The CPSB conducts intramural research in the areas of diet, nutrition and cancer, cancer chemoprevention, occupational cancer studies, and other cancer prevention strategies directed toward methods development and their application to reduce human cancer risk.

The **Laboratory of Nutritional and Molecular Regulation** (LNMR), located at the NCI-Frederick Cancer Research and Development Center (NCI-FCRDC), conducts a broad range of studies including the use of drug-resistance as a model for cellular defense, nutrient-dependent modulation of signaling mechanisms in cell proliferation, and dietary perturbation of nutrient and carcinogen metabolism.

The **Biomarkers and Prevention Research Branch** (BPRB) uses molecular biological techniques and clinical medicine to develop the biomarker tools for prevention and early detection of cancer. These programs are designed to improve the delivery and application of state-of-the-art cancer regimens.

All NCI intramural research is evaluated through peer review. Committees of outstanding scientists representing the various disciplines involved in the intramural research program periodically review the direction and progress of the research program and staff. All of the intramural program is subject to the same critical review, including new research directions concepts prior to their implementation. The committees that review the intramural research address the breadth and depth of each project and its relation to the Division mission. Critiques also address the quality, progress, future directions, and an assessment of resources and staff development. Recommendations made at the peer reviews are monitored and the impact of their outcomes are assessed in subsequent site visits by the DCPC Board of Scientific Counselors and its appropriate subcommittees.

In summary, the Division of Cancer Prevention and Control believes that a strong intramural research program complements the much larger extramural community. The program provides the facility to respond quickly to new directions, and to study and develop new paradigms for prevention and control research. The DCPC believes that an investment in intramural research is vital to the Nation's cancer prevention and control efforts. This Report outlines the program's progress and its promise.

BIOMETRY BRANCH

OBJECTIVES

The overall objectives of the Biometry Branch are summarized in the functional statement:

- "Plans and conducts independent and cooperative research studies on cancer epidemiology, prevention, screening, diagnosis, treatment, and control using methods of mathematical and analytic statistics;
- plans and conducts independent and collaborative studies in biostatistical and epidemiologic methodology and in mathematical modeling of processes relevant to cancer prevention and control activities;
- provides consultation and review of proposed projects concerning biostatistical methodology and study design to staff of the Division and to investigators in other divisions of the NCI and outside;
- provides expertise in statistics and biometry to program managers and scientific decision-makers within the NCI and outside."

OVERVIEW

The work of the Branch is conducted by its three Sections and by the Office of the Chief. The principal projects underway in each of these four organizational units will be described separately. The functional statements for each of the three Sections will precede the description of their projects. Projects that involve collaboration across Sections or with the Office of the Chief are described only once in this report to avoid duplication.

ACCOMPLISHMENTS AND PLANS

Office of the Chief:

Research in Statistical Methodology and Consultation for Cancer Prevention
(Z01 CN 00177-02 BB)

The Branch has two major roles within the Division: 1) conducting original research in statistical methodology, and 2) providing practical consultative services to the Division's entire intramural and extramural program. The Office of the Chief coordinates activities in a general sense, but the Section Chiefs are responsible for the specific activities of their staff. The projects listed below reflect the specific interests and activities of the staff within the Office of the Chief.

Women's Health Initiative (WHI) Clinical Trial

The staff have provided extensive consultation to a team of NIH scientists developing and implementing the WHI. This project involves research into prevention of diseases that are of particular concern to women: breast and colorectal cancer, heart disease, and osteoporosis. The initiative comprises three components: a clinical trial, an observational cohort, and a community intervention trial.

The WHI clinical trial will be one of the largest randomized prevention trials ever conducted. It will include 63,000 postmenopausal women from 45 clinical centers in North America. Three types of intervention for preventing women's disease will be studied: a low-fat dietary pattern that is high in fruits, vegetables, and fiber; hormone replacement therapy; and calcium with vitamin-D supplementation. Each intervention group of women will have a corresponding control group who do not receive that intervention. The design will be a partial factorial, in which eligible women will be offered the opportunity of participating in all three randomizations, with willingness to be randomized for either the dietary intervention or the hormone replacement therapy being required. Anticipated sample sizes for each randomization are 48,000 for dietary modification, 25,000 for hormone replacement therapy, and 45,000 for calcium supplementation, with sufficient overlap between the randomizations to allow a total sample size of 63,000. These sample sizes will provide good statistical power for detecting realistic reductions in the incidence of breast and colorectal cancer from dietary modification, heart disease from hormone replacement therapy, and osteoporosis from calcium supplementation. The coordinating center and 15 vanguard center contracts were awarded in 1992 and the trial is anticipated to start near the end of 1993.

The staff have developed methods and software for the estimation of statistical power and have carried out extensive calculations to design the clinical trial. The work involved adaptation and extension of previous work on incorporating partial noncompliance and weighted logrank tests into power calculations, as well as new work on incorporating the use of a weighted binomial test. Much of this work will form the basis of the statistical monitoring of the trial. Staff are now involved in development of the detailed monitoring plans.

Dietary Intervention for Patients with Colorectal Polyps (NCI Polyp Prevention Trial)

The staff has been collaborating with the Cancer Prevention Studies Branch in developing and implementing the NCI Polyp Prevention Trial. This major randomized study is designed to enter 2,000 patients from 10 clinical centers in the U.S. Half of the patients are allocated a dietary counseling program aimed at reducing their intake of fat and increasing their intake of fruits, vegetables, and fiber. The other half are allocated standard management. The patients will have had a colorectal adenomatous polyp removed shortly before entry to the trial and will be reexamined by colonoscopy at 1 year and 4 years after randomization. The main question to be answered is whether dietary change will reduce the recurrence rate of adenomatous polyps. The trial began at three vanguard centers in May 1991 and had recruited over 1,500 patients by the end of June 1993. Staff are involved with supervising the data collection and data entry, and with interim analysis and monitoring of the trial.

Validation of Intermediate Endpoints for Cancer Research

Because of the long duration from initiation to development of overt malignancy and the relative rarity of malignant disease, primary and secondary cancer prevention trials involve large numbers of subjects followed over many years. There is considerable interest in finding biomarkers which are intermediate endpoints for cancer and which may be reliably used as a surrogate for the cancer endpoint in a prevention trial. Even more importantly, discovery of such an intermediate endpoint will provide new clues to etiology and may lead to new approaches to prevention. Three branches of this work have been developed. First, statistical methods for developing and validating potential intermediate endpoints have been formulated, drawing together previous epidemiological work on causal inference and attributable proportion, and methodological work on surrogate endpoints in clinical trials. Statistical methods of validation have been described and illustrated using data on serum cholesterol and heart disease. This work has been used recently by the Food and Drug Administration for evaluating the use of CD4 levels as endpoints in AIDS clinical trials. Second, sample size requirements for validating intermediate endpoints in cohort studies or prevention trials have been addressed and the work

applied to the assessment of cell proliferation rates within an NCI dietary intervention trial among patients with colorectal polyps. Third, guidelines have been suggested for deciding when a biological marker may be used as an endpoint in Phase II trials of a new agent.

American Association for Retired People (AARP) Observational Cohort Study

Cohort studies for investigating the relationship between dietary intake and cancer depend upon the measurement of intake of nutrients by simple methods such as a food frequency questionnaire. It is known that such ascertainment methods give imperfect measures of the long-term average intake. This reduces the power of the study to detect dietary effects. The rather narrow range of dietary intakes in Western populations is another major factor for the very large sample sizes required to detect dietary effects. A two-stage sampling scheme has been developed in which a large sample of subjects is asked to complete a dietary questionnaire but only a subsample, selected to include preferentially those with extreme dietary intakes, is followed for disease incidence. This reduces the number of subjects requiring followup.

The development of this design has led to collaboration with the Cancer Prevention Studies Branch in the conduct of a large observational cohort study to further examine relationships of diet with breast, colorectal, and prostate cancer. The AARP is working with the Division to implement the two-stage design with members of the Association as cohort subjects. Subjects will be male or female, aged 50-69, and will be selected from one of five states that have comprehensive state cancer registries. Followup on 350,000 subjects will be conducted primarily through these registries. The concept for this study was approved by the Board of Scientific Counselors in October 1991, the contract was issued in 1992 and is now being completed.

Analysis of Data from Animal Chemoprevention Experiments

A statistical model, suggested by Kokoska (Biometrics, 1987), attempts to distinguish between the effect of a compound upon the number of tumors initiated in an animal at the time of carcinogen administration and its effect upon the time to appearance of the tumor. This model has been evaluated on several data sets. Experience shows that the results obtained are highly dependent upon the assumptions regarding the distribution of the number of tumors per animal. Goodness of fit tests have been developed to help guide the choice of distribution used in the analysis.

AIDS Research

Staff of the Biometry Branch are collaborating with staff of the National Institute of Allergy and Infectious Disease on several AIDS-related projects including an Observational DataBase (ODB) covering over 40 centers belonging to the Community Based Clinical Trials Network composed of members of the NIAID Community Program for Clinical Research on AIDS and AmFAR, the American Foundation for AIDS Research. Data collection began in October 1990 and over 13,000 HIV-positive individuals from have been entered into the AmFAR study as of July 1993. During the past year, the data forms for the AmFAR component of the study were extensively revised and detailed quality assurance evaluation was begun. The database will be used to permit rapid and accurate tabulation of descriptive data about HIV seropositive subjects potentially available for participation in randomized clinical trials and other studies and to generate hypotheses to be tested in randomized clinical trials.

Staff also collaborated with researchers in Toronto in the design and analysis of a trial of low dose oral interferon-α for persons with AIDS. This trial to evaluate the short term effect of interferon-α on CD4 cell counts and other measures of disease status found neither short-term benefits nor adverse effects from the therapy.

Analysis of Mammary Carcinogenesis Experiments in Rodents Related to the Effects of Fat

An analysis has been conducted previously of the many experiments in the literature investigating the effects of fat, calories, and mammary tumors in rodents. Work is in progress to evaluate the effect of the type of fat in these experiments. Results indicate that the linoleic acid content of the fat is an important factor in its effect upon mammary tumor development.

Bayesian Methods for Monitoring Trials

In long-term prevention trials or therapeutic trials, data on the effectiveness of an intervention become available before the end of the trial. It is often demanded, by ethical considerations, that data be analyzed to aid in the decision of whether to continue the trial as planned. The most popular statistical methods for aiding this decision are those which preserve the overall Type I error probability; they are known as group sequential tests. However these methods can be rather inflexible in practice and theoretically suffer from contravening the Likelihood Principle. An alternative method based on Bayesian theory has been developed and has been demonstrated to carry the principle advantages of group sequential methods—avoiding premature stopping—while retaining flexibility and conforming to the Likelihood Principle. These methods have been applied to data from important trials, such as the Levamisole and Flourouracil trial in patients with colorectal cancer, and have been shown to provide useful additional information for monitoring.

Calibration Studies of Food Frequency Questionnaires

Food Frequency Questionnaires (FFQ) provided a relatively quick and inexpensive means of assessing an individual's diet. However, without knowledge of the relationship between the FFQ report and the true usual intake of an individual, the results of studies that employ FFQs are not easily interpreted. Calibration studies are designed to allow estimation of this relationship. Individuals are asked to complete a FFQ (on one or more occasions) and a more detailed record of their diet, either a 24-hour recall or a multiple-day food record, on at least two occasions. Assuming the more detailed record to provide an unbiased measure of intake, we have developed a method of estimating the FFQ-usual intake relationship. Staff are applying this method to the data from calibration studies from the Women's Health Trial and the ATBC Finland Trial (Z01 CN 00100-11 CPSB). They are investigating the use of such methodology to interpret data from surveys that employ FFQs as their diet assessment instrument.

Pathology Review in Cancer Research

Little has been written about the design of pathology review studies. Staff have built on their previous research into the role of repented observations in pathology review to discuss broad issues in the design of such studies. They distinguish between reviews that are conducted to investigate the level of agreement between pathologists on a certain assessment, and those reviews that aim to determine the eligibility of patients to a research study. The design differences between the two types of study are highlighted.

Cancer Control Objectives and Cancer Mortality Projections (Z01 CN 00142-09 BB)

An interactive FORTRAN program that projects cancer mortality and incidence figures (numbers and rates) for forty years serves as a focus for several projects within the Division. The program incorporates two different survival models (Weibull and mixed exponential), over 40 cancer sites, the ability to begin with or without prevalent cases, temporal trends in underlying cancer incidence and in mortality from other causes, three possible types of intervention (primary prevention, screening, and treatment), age adjustment, calculation of annual incidence and mortality statistics, and comparison of these statistics under changing conditions of trends and interventions. The Branch staff work closely with the Surveillance Program and other DCPC staff. Work during the past year has included conversion of the program to the NIH's mainframe Convex computer and coordination with conversion of a similar program for personal computers.

Biostatistical Methodology and Cancer Control Epidemiology Section:

The overall objectives of the Section are summarized in its functional statement:

- "Plans and conducts independent and collaborative research concerning biostatistical and epidemiologic methodology related to the theory and analysis of cancer prevention and control studies;
- conducts or collaborates in the design and implementation of studies aimed at developing, refining, and testing hypotheses relating to applied cancer prevention and control, community oncology, and diffusion and adaptation of effective prevention, control, and treatment technologies;
- provides consultation to researchers both within and outside NCI on problems related to biostatistical methodology and epidemiology."

Consultation and Collaboration in DCPC Studies

In collaboration with the Applied Research Branch, data have been analyzed from a randomized clinical trial of isotretinoin for reducing the incidence of basal cell skin carcinoma (BCC) in a high-risk population. Nine hundred and seventy seven patients with two or more BCCs during the past year were randomly assigned to take either isotretinoin (13-*cis*-retinoic acid) or a placebo daily and followed at eight clinical centers. After 3 years the treatment was discontinued and the followup continued for another 2 years. Counts of BCCs observed at each 6-month return visit were recorded and formed the basis of a statistical analysis. Results show that the treatment has, at best, a weak (not statistically significant) effect on carcinoma incidence. Prognostic factors which were found to significantly predict BCC 6-month-visit incidence include: history of BCC incidence during a 5-year period prior to the study, a quantitative measure of solar damage to the skin, and the number of BCCs found at the previous 6-month visit.

In consultation with the Prevention and Control Extramural Research Branch, statistical advice is being given to the Worksite Health Promotion Intervention Study. Initial guidance was given on sample size calculations and pair matching of worksites necessary for the final statistical analysis. Possible difficulties from low response rates on the baseline survey are currently being examined and design of the final statistical analyses is being planned.

In collaboration with the Cancer Prevention Studies Branch, analyses are being conducted on the effects of micronutrient supplementation on immunology measures among subjects in the Linxian, China intervention study. Four hundred adults randomly selected for study from the nearly 30,000 subjects were supplemented with selected micronutrients for 5 years in a $\frac{1}{2}$ 2x4 fractional factorial design. Blastogenic responsiveness of T-lymphocytes to phytohemagglutinin-A (PHA) was determined in whole blood cultures. Males, but not females, supplemented with micronutrient combinations containing 120 mg vitamin C and 30 µg Mo showed lower ($P< 0.05$) T-lymphocyte blastogenesis than those not receiving them.

In collaboration with the Cancer Prevention Studies Branch, analyses have been conducted on the effects of alcohol ingestion on plasma levels of estrogens and androgens in 107 premenopausal women. Alcohol ingestion was estimated using a self-administered questionnaire on drinking patterns, a food frequency questionnaire and a 7-day diet record. Fasting blood samples were collected on days 5-7, 12-15, and 21-23 of each subject's menstrual cycle and pooled to create follicular, midcycle and luteal samples. Alcohol ingestion level was not associated with plasma estrogen, DHEAS or SHBG level in any phase of the menstrual cycle. However, it was found to be positively associated with the level of plasma androstenedione which is metabolized to estradiol in the breast.

In collaboration with the Cancer Prevention Studies Branch and the USDA, cross-over design feeding studies of the effect of daily alcohol intake on lipoproteins in premenopausal women have been analyzed. With alcohol, high density lipoprotein (HDL) cholesterol levels increased 9%, low density lipoprotein (LDL) levels decreased 8%, and levels of lipoprotein(a) were unchanged. The data from this study are also being used to estimate the differential effect of calories from alcohol and non-alcohol sources.

Vitamin E or tocopherol, a known antioxidant, may play a role in the etiology of chronic disease such as cancer and heart disease. A study examining the influence of "internal" (lipids, lipoproteins, and apoproteins) and "external" (dietary components, physical activity, body mass index) factors on plasma α-tocopherol and γ–tocopherol levels was completed and accepted for publication. For the non-supplement users ($n = 46$), plasma triglycerides and apoprotein were important determinants of plasma α-tocopherol levels, but these factors did not appear to affect levels in non-supplement users. Vitamin E intake was a significant "external" factor for both supplement and non-supplement users.

In collaboration with the Cancer Prevention Studies Branch, data from the Framingham Heart Study have been analyzed to evaluate the association between physical activity and breast cancer incidence. After adjusting for known breast cancer risk factors, the analysis found a moderate gradient of increasing breast cancer risk with increasing physical activity ($P = 0.06$).

In collaboration with the Black-White Survival Study Group, a comparison is being made of the treatment patterns among black and white patients with in situ or early stage breast cancer. Partial mastectomies were performed in 41% of the in situ cases, 28% of the Stage I cases, and 17% of the node-negative Stage II cases. Within stage of disease, there was no treatment difference between the races. Clinical tumor size and total family income were strong predictors of breast conserving surgery in both races while age and usual source of medical care were associated with type of surgery in whites only.

In collaboration with the Applied Research Branch, methods based on the Kappa statistic for measuring rater agreement are being developed to periodically evaluate the informational accuracy of the Cancer Information System. Design issues regarding the number of test calls to be taped, the number of raters to evaluate the tapes, and construction of the evaluation form are being considered.

In collaboration with the Cancer Prevention Fellowship Program, factors related to participation in a church-related cardiovascular disease (CVD) risk factor screening program have been analyzed. Worship service attendance frequency, age, knowledge of CVD risk factors, body mass index, smoking status, past history of elevated blood pressure, current blood pressure level, and residential distance from the screening site were all found to be significant predictors of participation in the program.

Extensive statistical consultation has been provided to the 5-A-Day Program. In the planning phase of the project, the Section was involved in the development of the baseline survey instrument and survey design, and in the development of sample weights. After the data were collected, adjustments were made for nonresponse. The possibility that the adjustments could be based on a propensity score modeling of the probability of response was explored. However, models using propensity scores were no more predictive of response rates than were models using standard post-stratification techniques based on demographic variables. Descriptive statistics have been produced, and fruit and vegetable intake has been modeled. Consultation was also provided on sample size estimation procedures for upcoming intervention studies in which communities would be the sample units.

Using food frequency data from the 1987 Cancer Control Supplement to the National Health Interview Survey (NHIS), a comparison of eating patterns of whites, blacks, and Hispanics, undertaken in collaboration with the Applied Research Branch, shows both similarities and differences in consumption of a large number of the foods across these ethnic groups. Such differences may have implications for public health nutrition education programs. For every food or food group, the proportion of non-consumers was determined as well as the median intake and the interquartile range. These data suggest that women had a more varied diet than did men. Women reported consuming more fruits and vegetables, less meat, and fewer high-fat foods than did men. Whites appeared to eat a greater variety of foods then did blacks or Hispanics. Blacks reported consuming more fried and high-fat foods then did either whites or Hispanics; consumption of high-fat foods was lowest among Hispanics. Based on these findings, it was concluded that public health messages can be targeted at increasing the overall consumption of fruits and vegetables, decreasing consumption of high-fat foods, especially among white and black men, and increasing consumption of healthful foods shown to be popular overall or among particular race/ethnicity groups. A similar analysis of dietary patterns of Cuban, Mexican, and Puerto Rican Americans is underway. In addition to intake of individual foods, nutrient intake is also being examined.

Four-day diet record data on some 2,500 women interviewed in the 1985 and 1986 Continuing Survey of Food Intakes by Individuals (CSFII) are being used to characterize the frequency of consumption of a variety of foods and food groups, in a project in collaboration with the Applied Research Branch. Foods have been categorized for analysis and are being coded; for example, consumption of vegetables both raw and cooked, separately or in mixtures, and both with and without added fat, will be studied.

Consultation and Collaboration in Studies Outside DCPC

In collaboration with Epidemiology & Biostatistics Program of the DCE, an analysis has been made of dietary risk factors in a case-control study of lung cancer among nonsmoking women. The primary finding is that saturated fat intake is strongly related to increased cancer risk. Intake of beans & peas and citrus fruit & juice are also significantly related to risk, but more weakly. Saturated fat is most strongly associated with incidence of adenocarcinoma rather than with other histologies.

In collaboration with the Threshold Limit Value Committee of the American Conference of Governmental Industrial Hygienists, an analysis was conducted of leukemia mortality among the Pliofilm workers exposed to benzene during 1940-60. Our risk estimates are different from those found by earlier analyses. The primary difference is because we used a different dose-response model which 1) provides a better fit to the observed data, 2) agrees with laboratory and animal studies showing benzene metabolism to have saturable kinetics, and 3) leads to lower "acceptable" occupational exposure limits.

In collaboration with the Environmental Protection Agency, a study to determine which characteristics of human male semen are predictive of fertility potential is being analyzed. Over 200 couples without risk factors for infertility who were willing to attempt pregnancy enrolled in the study. They were closely followed for 3 complete menstrual cycles. Women were counseled on optimal coital schedules and post coital testing was performed to insure compliance. Semen samples were obtained during or just after menses in each cycle and just after the 3rd cycle or when pregnancy was confirmed. Pregnancy outcome was followed for another 9 months or until a positive result. Using a Cox regression, the numbers of total sperm and motile sperm were significantly related to pregnancy outcome. A sample of approximately 200 sperm from each semen sample are planned to be analyzed in detail. A more detailed analysis of the relationship between characteristics of these sperm and time to pregnancy will then be conducted.

Research in Biostatistical Methodology and Mathematical Modeling (Z01 CN 00121-09 BB)

Energy Adjustment Methods for Nutritional Epidemiology

Different statistical methods which adjust the effect of macronutrient intake for total energy intake are currently being used to analyze epidemiologic studies of diet and disease. This research project is examining the statistical properties of these methods. During the past year the project has accomplished the following:

1) accepted for publication was a paper which interprets the regression coefficients of three alternative regression models which measure nutrient intake; we show that four different effects of interest (related to either adding calories to the diet or substituting sources of calories in the diet) are estimable by each model, that these effects have important public health implications and we derive the standard errors of these estimates;

2) conditionally accepted for publication was a paper which examines the behavior of these methods when the study subjects are categorized into a small number (3-5) of groups according to their nutrient intake; a general theory of mean square linear stochastic regression has been developed for the case when stochastic explanatory variables are misspecified; the theory, supported by simulations, has been applied to energy-adjustment methods to examine the effect of categorization in four models, when the nutrient intake is either categorized into quartiles or ordered so as to investigate trend over the quartile groups, combined with using an adjusting variable that is either left continuous or categorized into quartiles; when the true macronutrient intakes and their inter-relationships are known without error, one result of this investigation showed Willett's "residual" method to be more powerful than the "standard" method (both measuring the effect of calorie substitution) and very similar to the "density" method.

Current activities of this research project are evaluating the effects of 1) random and systematic errors on measuring nutrient intake and 2) incorrect assumptions concerning inter-relationships among the true nutrient intakes. To study the consequences of measurement errors on the interpretation and properties of energy-adjustment methods, a theory of linear stochastic regression with measurement errors for the independent variables has been developed for the general case where the measurement errors correlate among themselves and with the true values of the explanatory variables. The theory allows one to calculate the expected value and variance-covariance matrix of Ordinary Least Squares estimates.

Future research directions include 1) determining conditions under which the "residual" and "density" methods give similar results and 2) evaluating the costs and benefits of using categorization to guard against model misspecification.

Stepwise Regression Issues

Many regression procedures which have found widespread application in biostatistics involve multi-step model building based on the use of repeated tests of significance. The theory has been developed that addresses problems resulting from repeated testing for a sequential forward selection procedure. It is shown that the commonly used F-ratio does not have the conventional F-distribution at any step of the procedure beginning with the second one, but an appropriate conditioning helps in developing the "correct" testing procedure.

Application of exploratory analyses for selecting the "best" regression predictor model affects statistical properties of conventional Mean Square Error of Prediction estimators and, in particular, can lead to their substantial bias. Different bootstrap-type estimators (both parametric and nonparametric) that might allow for the selection effect have been studied using theory and Monte-Carlo simulations. It appears that although the direct application of the bootstrap idea does not produce good results, some modified bootstrap estimators have much better statistical properties than the conventional ones and can be successfully applied in practice.

Cancer in Oriental Populations (Z01 CN 00113-10 BB)

Mainland China – 65 County Study

An in-depth diet, lifestyle, and mortality survey of 65 mostly rural counties has been conducted by researchers in China and elsewhere. As part of the study, an ecological survey in 1983 included details on nutrition and lifestyle through use of a questionnaire, food composition analysis, three-day diet survey, and blood and urine analysis. In cooperation with researchers in China, the findings were made available to us, together with 1975 mortality data, for an analysis on selected causes of death.

We have correlated various measures (nutritional status, reproductive history, etc.) with several components of cardiovascular diseases. We noted several statistically significant relationships among the mainland Chinese that have also been observed in the U.S., such as negative associations of cardiovascular disease with oleic acid (unsaturated fat), liquor (at lower or moderate levels) and legumes, and positive associations with salt, triglycerides, and herpes virus infection. Other associations not previously noted were negative correlations of molybdenum and age at first pregnancy, both also negatively related to certain neoplasms.

Asian Resource Data

A statistical file of age-specific and age-adjusted (using several different standards) incidence and mortality rates for cancer/noncancer causes since 1960 has been completed, covering the Chinese, Japanese, and Filipinos in the U.S. and "home" countries. These data will provide background information on the health status of the Oriental populations in matters of hypothesis formulation and program planning.

Design and Analysis of Pharmacokinetic Studies of Selenium (Z01 CN 00107-11 BB)

Selenium is a possible cancer preventive agent and is being considered for use in intervention trials. A study in collaboration with the Cancer Prevention Studies Branch is in progress that will provide information on the pharmacokinetics of selenium in its prototype forms—sodium selenite (inorganic form) and selenomethionine (organic form). This information is necessary for the determination of time and manner of administration. In the study, 32 subjects received a single oral tracer dose of selenite or selenomethionine on two occasions, 90 days apart, once fasting and once nonfasting.

An objective of the study was the comparison of pharmacokinetic parameters in fasting and nonfasting subjects. A kinetic model of selenite metabolism, developed as part of this project, has been used to analyze tracer data for each subject in both fasting states, taking into account both tracer indigenous in the diet and tracer from the first dose remaining in the body when the second was given. In previous years of this project, analysis of selenite tracer data suggested that fasting status modulated the effects of the first plasma component. While there was no biologically significant difference in absorption between fasting and nonfasting states, there was a greater first pass effect in nonfasters, probably in response to eating. Many parameters (e.g., delay time in the liver) change with fasting state, while others (e.g., proportions of material passing into the bile) do not change. Such information is important in deciding on an optimal dosing regimen. However, the model was found not to predict known body burden or masses of material in the liver. As an approach to this problem, a loss pathway, which takes account of losses from the skin, sweat, and breath, is being incorporated in the model; subject-specific adjustments for dietary intake and body load are being calculated.

A model for the metabolism of selenomethionine has also been developed. These models suggest that there are important kinetic differences between selenite and selenomethionine. Selenium from selenomethionine was better absorbed and retained than selenium from selenite, and the whole-body turnover was greater. In contrast to selenite, which is excreted after turnover

in the peripheral tissues, selenomethionine is reutilized. If recycled material is incorporated into metabolically active species, this reutilization could be advantageous and may have implications for cancer control. The two models are being modified so that they can be combined into a single model that will better simulate our dietary intake of selenium, which includes both inorganic and organic material.

A workshop, "Selenium Compounds in Cancer Chemoprevention Trials," is being organized jointly by the Biometry Branch and the Chemoprevention Investigational Drug Unit. The workshop is being held in anticipation of the possible expanded use of selenium compounds as chemopreventive agents. Leading selenium researchers have been invited to give presentations providing an overview of the current state of selenium research and to participate in roundtable discussions aimed at obtaining information necessary to submit NDAs (New Drug Applications) to the FDA.

Clinical and Diagnostic Trials Section:

The overall objectives of the Section are summarized in its functional statement:

- "Engages in independent and cooperative research on statistical methodology for design of controlled clinical trials of cancer prevention and treatment, and for field testing of diagnostic techniques;
- provides full statistical support in selected trials, including development of the detailed study plan, supervision of data collection, processing, and editing, and analysis of the data as well as preparation of scientific papers;
- develops statistical techniques for analyzing trial results, for identifying prognostic factors and diagnostic determinants, and for analyzing observational data;
- consults and collaborates extensively with other researchers requiring expertise in these and related areas."

Statistical Methodology Research (Z01 CN 00116-10 BB)

Methods for Analyzing Complex Survey Data

Data from household surveys such as the National Health and Nutrition Examination Survey (NHANES-I) followup and the National Health Interview Survey (NHIS) derive from clustered samples of persons that are usually selected at differential rates. These aspects of the sampling result in nonindependence and unequal weighting of the observations that should be considered during the analysis stage. Survey data are used extensively in three types of study designs: 1) cohort studies through long term followup of the sample, 2) case-control studies by providing population controls, and 3) cross-sectional studies. An estimating equation approach along with robust Taylor linearized variance estimation has been developed for logistic regression of case-control studies when the controls come from a cluster sample. This investigation concluded that under cluster sampling 1) the classical estimating equations are more efficient than other weighted estimating equations (e.g., when the weights are the inverse of the probability of selecting controls from the population), and 2) the loss in efficiency from cluster sampling decreases when the control to case ratio is increased. Simulations have been used to quantify the inefficiency of the weighted estimating equations. A data example from an analysis of the 1987 NHIS and 1986 National Mortality Followback Survey on oral cancer and alcohol consumption has been used to illustrate the estimating equation approach.

Procedures have been developed that involve trimming sample weights and modeling the complex sample designs of surveys so as to make analysis more efficient . These procedures have been applied successfully to analyses from NHANES-I, NHANES-II, and the Hispanic HANES. Statistical tests relying upon chi-squared and F distributions have been developed for testing whether these procedures are consistent with the data. Simulations have shown that the distributional assumptions of these tests are not always valid because of the complex sample design; however, the levels of these tests are still nearly correct.

The Section is collaborating with statisticians at the National Center for Health Statistics on a large simulation to study the empirical properties of jackknife, balanced-half-sample, and Taylor linearized variance estimation procedures, and level and power properties of Wald tests and design-effect-adjusted Wald tests. Data from the 1979, 1980, and 1981 NHIS are being used as the finite population from which repeated samples are selected under a variety of different sample designs. Preliminary results have shown that the balanced-half-sample replication method had sizable bias when estimating ratios for small subdomains, while jackknife and Taylor linearized variances remained nearly unbiased. The results from this project will offer guidance to survey designers and analysts about the best statistical methods to apply to complex survey data.

Analysis of Diet Survey Data: Typical Consumption and Effects of Covariates

A parametric statistical model was constructed to model count data provided by 24-hour recall questionnaires. Observed counts are assumed to follow a mixed Poisson distribution whose parameters involve observed covariates. The model applies to populations with a mixture of abstainers and consumers, and interindividual variation is modeled using the gamma distribution. The model permits one to relate abstention and average consumption to covariates, such as income, race, day of week or season, using regression models. An important feature is that the model separates within and between person variation of count data. As a result, one can estimate the distribution of typical individual consumption, either for the entire population or for selected subpopulations.

The parameters of the model are estimated by maximum likelihood, enabling the analyst to test hypotheses or to create confidence intervals using large sample theory. Simple graphical techniques to assess model validity were also devised. Weighted likelihood techniques permit the model to be applied to data from complex sample surveys. The finite sample properties of the procedures were assessed using simulation, and are in agreement with theory when the model assumptions hold. The model was also applied successfully to count data extracted from the 1985 Current Survey of Food Intake by Individuals. The results conformed to findings of other investigators, indicating that the model has practical value in analyzing count data from future dietary studies.

This methodology can be applied to problems other than food consumption surveys. Data from animal studies of teratology or familial data where the response is a count can be analyzed using this model. The technique can also be generalized to problems with binary or continuous responses.

Computer Models of Population-Based Cancer Screening Programs

Computer models are an essential tool for predicting cancer incidence and mortality and for estimating the costs and benefits of improvements in cancer detection, prevention, and treatment. The NCI is currently engaged in refining these computer models and adapting them to run interactively on modern workstations.

As part of this effort, in conjunction with the Applied Research Branch, a new model for assessing the effects of screening was developed and programmed. The basis of the model is a Markov chain representation of the progression of cancer from early to intermediate to advanced stage disease. The model tracks prevalence of undetected disease and incidence of symptomatic disease for a hypothetical population, by age and stage of disease. When screening is introduced in the population, the model computes age and stage specific screen detection and symptomatic detection rates.

Because the model represents the natural history of disease, it is capable of reproducing observable findings in actual screening trials, such as an increase in incidence when screening begins and an increase of early stage disease relative to advanced stage disease. The model accommodates age-specific incidence and the effects of false positive and false negative screening results. Actual parameter values can be estimated from large-scale screening trials, such as the HIP study of breast cancer screening or the PLCO trial of prostate, lung, colorectal, and ovarian cancer, which is currently in the advanced planning stages.

Analyzing Disease Progression with Status Measurements at Fixed Time Points: Use of Permutation Tests

In intervention trials in which patient status is measured regularly as an indication of disease progression, we often want an overall measure of impairment over time. Such measures are particularly of interest in the absence of a specific irreversible event whose incidence or time-to-occurrence can be compared between groups. Examples are measurements of performance status for cancer patients, and measurements of visual function in eye disease. In such situations, although disease tends to progress, status can improve between one visit and the next (whether real or apparent). These measures can be compared between randomized treatment groups as a study outcome, or between categories of baseline variables when exploring prognostic factors.

An approach to this problem has been explored, using permutation tests to determine statistical significance. In this approach, each person contributes a certain number of person-visits. At each visit, we determine the decrement in status from baseline, either quantitative or binary (i.e., based on whether it meets the criteria for an important decrease). These are averaged for each group over all person-visits, and the difference between groups is calculated. For the permutation test, we take the individual sequence of outcomes as a unit (with missing values), and randomly assign to group; recalculate the statistic for each set of random assignments; and the p-value is percentage of these that equal or exceed the observed value. The structure of the study (e.g., pair matching) needs to be taken into account in these calculations.

Comparison of Methods for Identifying Prognostic Factors and Predicting Survival for Patients with Colorectal Cancer

In collaboration with a working group of the American Joint Committee on Cancer, work is underway on fitting Cox proportional hazards models to a dataset on patients with colorectal cancer. The goal is to identify important prognostic factors and then apply these to predict survival probabilities at various points of time. The models are being fitted on one-half of the dataset and then used for predictions on the other half. The success of these predictions are being compared with three other methods used by other members of the working group. The other methods are recursive partitioning, Bayesian analysis, and neural networks. Various measures of success of prediction are being investigated for comparing the four methods of analysis.

Interactive Statistical Programs

The Section has previously developed and continues to maintain and improve a group of interactive computer programs for efficient analysis of medical data, particularly those dealing with risk factors and prognostic factors using sophisticated multiple regression techniques and

survival analysis. These programs have proven useful not only for many projects within the Biometry Branch but also elsewhere in the Division, as well as by other investigators both within the NIH and at outside institutions.

Sample size and power calculations have been implemented for use in studies where a group is the unit of randomization and analysis. The formulae in this computer program have applications for computing power for smoking and dietary intervention studies, such as the Community Intervention Trial for Smoking Cessation (COMMIT) and the 5-A-Day Program.

Consultation on Clinical Trials and Other Studies (Z01 CN 00119-10 BB)

Community Intervention Trial for Smoking Cessation (COMMIT)

Extensive consultation has been provided to the Prevention and Control Extramural Research Branch concerning statistical issues that have arisen in the planning and implementation of COMMIT, a large-scale community-based study intended to promote smoking cessation among heavy smokers. Staff of the Biometry Branch have devised the basic design for the study—eleven matched pairs of communities with one member of each pair chosen at random for intervention and the other serving as a control. The study was designed to detect a 10% difference in the smoking quit rate between the intervention and control communities. Biometry Branch staff, with the lead taken by the Clinical and Diagnostic Trials Section, have been actively involved in all meetings of the Steering Committee, and have analyzed and presented interim data to the independent Policy Advisory Committee.

The COMMIT intervention ended in 1992, and the final surveys have been implemented. The Branch has been actively involved in survey design and content. These final surveys are:

1) Endpoint Cohort Survey—The full cohorts of heavy and light-to-moderate smokers were contacted in the spring of 1993 to determine the self-reported, six-month cigarette smoking cessation rates in each of the 22 communities. This measure is the primary endpoint of the COMMIT trial and will be used to determine the COMMIT intervention effectiveness in increasing smoking cessation.

2) Cotinine Validation Study—After the completion of the Endpoint Cohort Survey, the "quitters" in the heavy smoker cohort will be re-contacted for participation in this study. Saliva samples will be collected from eligible and consenting participants for cotinine measurement. The study is designed to measure cessation misrepresentation rates between the COMMIT intervention and comparison communities.

3) Evaluation Cohort Survey—Cohorts of 400 adults from each of the 22 communities were contacted a third and final time in the spring of 1993 to measure the population-wide impact of COMMIT on intervention awareness, participation, and the decline of the social acceptability of smoking.

4) Final Prevalence Survey—A cross-sectional sample of 3,000 adults in each COMMIT community will be surveyed to determine adult smoking prevalence. The survey will be conducted in the autumn of 1993 and will provide a measure of the trial's secondary endpoint, smoking prevalence.

5) Youth Survey—A random sample of 400 ninth grade students were surveyed in each community in autumn 1992 to measure smoking knowledge, attitudes, and behavior.

6) Religious Organizations, Worksites, and Physicians Offices Surveys—Random samples of each were underway in the summer of 1993 to assess the implementation of no-smoking policies, adherence to policies, and participation in smoking cessation activities.

7) Cessation Resources Survey—Organizations involved in health promotion activities were surveyed in 1993 to determine the level of participation in anti-smoking activities and smoking cessation programs.

8) Physicians and Dentists Survey—Health professionals were surveyed in 1993 to measure the level of patient counseling for smoking cessation and participation in formal training for smoking cessation counseling.

The Branch has been planning the statistical analyses for the major COMMIT endpoints. This has involved the exploration of various statistical techniques for adjusting observed differences using baseline prognostic factors, and for handling missing data using baseline covariates and patterns of intermediate endpoints. Computer simulation studies have been used to study the properties of various approaches. This has aided in the selection of procedures being applied to the data from the final Endpoint Cohort Survey.

Brain Tumor Clinical Trials

The Section provides full statistical support for the Brain Tumor Cooperative Group (BTCG), a multicenter group of neurosurgeons, neuro-oncologists, radiotherapists, neuro-radiologists, and neuro-pathologists conducting randomized trials for patients with primary brain tumors (with emphasis on malignant gliomas). The BTCG has continued to accrue patients to a randomized phase III trial, BTCG 87-01, investigating interstitial radiation (seed implants) as an addition to the customary external beam radiation and chemotherapy, and further interim analyses of the data have been performed.

Accrual also continues on another randomized trial, BTCG 89-01, that compares two phase III chemotherapy regimens to be given in addition to surgery and radiotherapy. One regimen is the standard intravenous BCNU; the second is the combination of intravenous BCNU with intra-arterial Cisplatin. The trial had also included a third arm in the randomization, used to investigate, successively, new investigational phase II drugs. The initial agent was 10-EDAM (Edatrexate). When accrual for this group was completed in early 1992, randomization was begun to Piroxantrone. During the past year, accrual to this third arm was terminated early by the Division of Cancer Treatment (which funds the BTCG and is responsible for all official NCI decision-making concerning these trials), because of a decision not to pursue the agent Piroxantrone. Followup continues on all patients on BTCG 89-01, with appropriate data monitoring, and accrual continues to the first two arms.

BTCG 87-30, a study for patients with low-grade glioma, has been terminated, due to low accrual coupled with past decisions by the other two participating cooperative groups to discontinue participation. Followup continues on patients who had been entered on study.

Design Issues in Large-scale Prevention Trials

The Section has been involved in consultations on several large-scale, randomized trials for cancer prevention, in collaboration with the Early Detection and Community Oncology Program. One of these is the NCI-sponsored randomized trial of tamoxifen for preventing breast cancer (Breast Cancer Prevention Trial), to which accrual began in May 1992. Issues here have included projecting the probabilities of developing breast cancer for specific categories of women at high risk, and issues in data monitoring for a trial with multiple types of endpoint events. Other consultations have dealt with design of the upcoming randomized trial of chemoprevention of prostate cancer with finasteride (Proscar).

Analysis of USDA Feeding Studies

The Section has been collaborating with the Cancer Prevention Studies Branch on the analysis of a series of USDA feeding studies of men and women 20-40 years of age. Research has been completed for a sample of men, which involved a correlational analysis of intakes of the five major carotenoids from food frequency, seven days of diary records, and blood levels. Only modest diet-plasma correlations were found for the five carotenoids. Similar analyses have been conducted among a sample of women. Preliminary results have shown larger diet-plasma correlations than were found for the men, but the magnitudes of the correlation varied among the specific carotenoids.

Study of the Adoption and Use of the Primary Care Nutrition Guide

The Section has provided statistical consultation to the Public Health Applications Research Branch on the design, planning, and implementation of a physician practice study evaluating the NCI Primary Care Nutrition Guide and a training course (based on the Guide) among internal medicine and family medicine practices in Pennsylvania and New Jersey. Physician practices have been randomly assigned, according to a three-arm design, to receive the Guide with training, receive the Guide without training, or get neither the Guide nor training. About six months after initiation of the intervention, the physicians in the study and their staff will be interviewed to determine their knowledge and behavior about nutrition; the responses in the first arm will be compared to those in the second and third arms. Issues dealt with during the past year have included random subsampling of physician practices from the three arms because of over-recruitment, specifications of the study plan to include an intention-to-treat analysis, identification of variables to be used for evaluating the level of effort to recruit and interview physicians, and operational decisions for maintaining the integrity of the randomized design. The Section is planning to conduct the data analysis for this study.

Screening Section:

The overall objectives of the Section are summarized in its functional statement:

- "Plans, conducts, and analyzes independent and cooperative research studies in screening for the early detection of cancer;
- conducts methodologic research in statistics, probability, and epidemiology with particular emphasis on techniques appropriate to the design, analysis, and modeling of randomized and observational studies in cancer screening and related areas;
- engages in independent and cooperative research to determine cancer how the natural history and risk characteristics of populations apply to the design and interpretation of early detection and related studies;
- maintains liaison with other agencies, organizations, and professional societies concerned with cancer screening and related methodology in order to coordinate and optimize activities."

Studies in Cancer Screening (Z01 CN 00106-11 BB)

Data from several cancer screening studies are being collected and analyzed to gain a better understanding of the impact and consequences of such screening in various population settings. Staff are involved in design, monitoring, and data analysis aspects of these studies. The results can be used by the NCI in establishing cancer control policy. These databases also provide an opportunity for the development and testing of new techniques for data analysis.

Screening Trial for Prostate, Lung, Colorectal and Ovarian Cancer—the PLCO Trial

During the past year the staff of the Screening Section has been in continual collaboration with the Early Detection Branch and the Research Contracts Branch in developing the three major components of the PLCO Trial, namely the Study Coordinating and Data Management Center, the Screening Centers, and the Laboratory to perform blood testing. This is a major trial of cancer screening in males and females for four cancers that comprise more than 50% of the incidence and mortality of cancer—lung, prostate, colorectal, and ovarian cancers. The trial design calls for a total sample size of 74,000 males and 74,000 females between the ages of 60 and 74 who are to be divided at random into two groups. One group will be screened for prostate, lung, and colorectal cancers among males and lung, colorectal, and ovarian cancers among females, while the other group will serve as a control. The screening techniques to be used are annual digital rectal examination and prostate specific antigen for prostate cancer; annual chest film for lung cancer; three-yearly flexible sigmoidoscopy for colorectal cancer; and annual ovarian physical examination, CA-125 marker, and transvaginal ultrasound for ovarian cancer. Contractors were chosen for the Coordinating Center, ten Screening Centers, and a Laboratory. Meetings were held with the investigators to develop the final protocol, obtain OMB clearance for study forms, and prepare staff and facilities at the Screening Centers. Entry of study participants and initiation of the Pilot Phase of the trial is planned for October 1993.

International Working Group on Information Systems in Breast Cancer Detection

In December 1988 an International Workshop on Information Systems in Breast Cancer Detection was held in Rockville, Maryland under the sponsorship of the U.S. Food and Drug Administration (FDA) and the NCI. Screening Section staff collaborated with officials at the FDA to organize this Workshop. Participants came from Australia, Canada, Finland, Iceland, Italy, Hungary, the Netherlands, Sweden, the UK, the USSR, and the U.S., and included representatives of the World Health Organization and the International Union Against Cancer (UICC). Discussion was directed primarily at 1) developing a better understanding of how breast cancer detection is evolving in practice in different countries, and 2) initiating a process for the development of a database containing key data elements from each country that could be used jointly or individually by the countries for evaluation of breast cancer detection.

The papers presented at the Workshop have been edited, supplemented with recent information, and compiled into a proceedings volume that will be published by Hogrefe and Huber Publishers. The process that was started at the Workshop is being continued through a Working Group of the participants and focuses on development of a uniform, minimum data set and methods for measuring changes on a national or regional level. Screening Section staff are collaborating with members of the Applied Research Branch as well as investigators at the FDA and Johns Hopkins University in this Working Group. An initial database questionnaire has been developed and definition of terminology has been refined. Initial collection of data was begun in several countries and several alternatives for data collection in the U.S. were considered.

Other Studies

Three large-scale randomized trials have been conducted by the NCI to evaluate screening for breast, lung, and colorectal cancer. Staff participate in the analysis of completed studies and conduct of ongoing studies. The database from the Health Insurance Plan breast cancer screening trial was used to address several scientific and modeling issues. This study demonstrated a 25% reduction in breast cancer mortality after 10 years as a result of screening with physical examination and mammography, and has served as the basis for NCI policy and studies in other countries. Analysis focused on the magnitude and duration of the benefit, age-specific effectiveness, and application to model development and validation. Incidence and mortality data from the lung cancer screening trials conducted at Johns Hopkins University, Memorial Sloan

Kettering Hospital, and the Mayo Clinic were analyzed. The colorectal cancer screening trial at the University of Minnesota is currently in progress to evaluate testing for occult blood in the stool as an early detection maneuver for colorectal cancer. The trial reported a 32% reduction in colorectal cancer mortality in the screened arm. Staff participate in scientific consultation and ongoing data monitoring for this study.

Neuroblastoma is the most common solid tumor in children under age five, and interest has recently increased in screening for the early detection of this lesion. A consultative effort was continued with investigators at the University of Minnesota who are coordinating a controlled study to evaluate screening for neuroblastoma. The test procedure involves measuring the urinary catecholamine metabolites vanillylmandelic acid and homovanillic acid in specimens from infants in Quebec, Canada. Control populations are drawn from the state of Minnesota, the Greater Delaware Valley, and the province of Ontario.

Research in Cancer Screening and Statistical Methodology (Z01 CN 00105-11 BB)

The focus of this project is the development and refinement of statistical procedures for the design and analysis of cancer screening and related studies. Problems under investigation include an examination of analysis methods and endpoints for screening studies, assessment of case-control and other study designs for screening evaluation, development of methods to estimate benefit and bias in screening studies, derivation of novel approaches to the analysis of categorical data, and geographic analysis and smoothing of cancer mortality rates. Each of these problem areas is common to screening and prevention studies in which the Division participates, but the methods for screening studies must address the special lead time and length biases inherent in screening programs.

Mortality Analysis of Screening Randomized Trials

The analysis of a cancer screening randomized controlled trial in which there is appreciable followup after the trial's screening intervention has stopped is difficult because the effect is diluted after screening ceases. This project considers the Overall Analysis involving all the individuals randomized and a Limited Analysis based on subgroups of individuals with cancer diagnosed during defined periods from entry into the study. Statistical testing procedures are presented and illustrated for the two analysis approaches.

The determination of comparable case groups is critical to the validity of the Limited Analysis. Procedures to assess comparability of subgroups of cancers are investigated. The cancer incidence and covariates that reflect the natural history of the cancers are considered. First, the cancers in the subgroups being considered as possible comparable groups to be used in the Limited Analysis are examined. Second, the cancers that arise after these candidate groups are defined are investigated for their comparability. If these are not comparable, the candidate cases cannot be. This consideration of the late cancer groups has not been explicitly suggested previously.

Dilution of Effect in the Mortality Analysis of Cancer Screening Randomized Trials

The mortality analysis of a cancer screening randomized controlled trial (RCT) in which there is appreciable followup after the trial's screening intervention has stopped is complicated by the dilution of the screening effect that occurs after screening ceases. A simple model is developed to demonstrate and to estimate the dilution that can occur depending on the analysis done. Two analyses are considered: an overall one based on all those randomized to the RCT, and a limited analysis based on a restricted set of cancers diagnosed in the RCT.

Case-Control Studies

This project has focused on the ability of the case-control study to provide estimates of the efficacy of screening for those screened where efficacy is defined to be the mortality reduction of those screened relative to their mortality in the absence of screening. In the usual setting the case-control approach provides an estimate of the mortality reduction of those offered screening and being screened relative to those offered screening and not being screened. It was demonstrated that this, in general, results in a biased estimate of the efficacy, and may overestimate or underestimate the true impact of screening.

Currently the MISCAN cancer screening model is being used to simulate case-control studies of cancer screening. This project is a collaborative effort with investigators at Erasmus University, Rotterdam, the Netherlands, who developed the MISCAN model. Output from several runs of the MISCAN program has indicated that there are problems with bias if the usual or standard procedures for selecting controls and for defining exposure to screening are followed. Thus, current research is intended to assess the magnitude and direction of these biases in the results of a case-control study, both with and without a benefit from the screening, and under various levels of compliance.

A New Design for Evaluation of Cancer Screening

Research is continuing on a new design for screening randomized trials in which subjects in the intervention group receive repeated screens throughout the screening period and subjects in the control group receive one screen at the end of the screening period. The analysis is then confined to cancer cases ascertained in both groups up to that time. The purpose of this design is to reduce costs by decreasing the number of subjects requiring long term followup.

The Paired Availability Design

The paired availability design (PAD) is a new method for evaluating a treatment when a randomized trial cannot be performed. The PAD has three fundamental characteristics: 1) the intervention is the availability of treatment, 2) the population from which subjects arise is well defined with little in- or out-migration, and 3) the study involves many pairs of control and experimental subjects. By focusing on the availability, and not the receipt, of treatment, and with little migration into or out of the population, PAD reduces the possibility of selection bias. By using many pairs, PAD averages the effect of chance fluctuations in response which are associated with each pair.

Composite Linear Models

A composite linear model (CLM) is a matrix model for incomplete multinomial data. A CLM provides a unified approach for maximum likelihood inference which is applicable to a wide variety of problems involving incomplete multinomial data. By formulating a model as a CLM, one can simplify computation of maximum likelihood estimates and asymptotic standard errors. An important application of CLMs has been the estimation of sensitivity and specificity for various modalities for the early detection of prostate cancer. Another application is the analysis of repeated incomplete categorical data. This includes cross-over trials and longitudinal studies. Potential applications include the analysis of replicate observer agreement data, and the analysis of case-control data with a validation sample or a two-stage sampling design.

Monitoring Cancer Screening Trials

It has become common practice to use monitoring procedures during the course of clinical trials. Such practices inform investigators of any adverse effects, as well as of any substantial early benefits of the intervention that would lead to early termination of the study. Existent

monitoring procedures have largely been developed in the context of therapy trials, especially in the cancer field. Monitoring for adverse effects of therapy is clearly of importance in cancer therapy trials, particularly when cytotoxic drugs are being tested. In a cancer screening trial, on the other hand, it is thought unlikely that the screening test will increase mortality. The key question in cancer screening is whether an early detection procedure can lead to reduction in mortality from the cancer of interest.

Because these trials typically involve large populations, and last ten to fifteen years or more, it is important to monitor such trials closely to ensure that such trials are proceeding according to protocol. It may be that many monitoring techniques developed for therapy trials apply to the screening setting with little modification, or alternatively, that new approaches specific to screening trials must be developed. To date little has been done in this area. This work is intended to explore these issues for cancer screening trials. Monitoring variables under examination include compliance, prevalence and incidence, screening test properties, cancer case characteristics, survival data, and mortality data.

Estimation of Lead Time and Screening Benefit in Randomized Cancer Screening Trials

The increased survival time of screen detected cases in a randomized screening trial over those that are detected clinically may be due in part to lead time, or the length of time by which the disease is diagnosed earlier by screening in the absence of any real extension in survival time. How much of the observed survival time among screening participants with cancer is lead time, and how much is actual benefit time, i.e., the length of time by which survival has been extended beyond merely the time of the advanced diagnosis? An accurate determination of these two components of observed survival time may have important consequences for policy decisions regarding frequency and necessity of screening, particularly in diseases such as prostate cancer where a screening test is available but its impact on the general population being screened is unknown. A methodology is proposed whereby both benefit time and lead time may be estimated directly from randomized screening trials. The methodology involves examining the difference in survival from time of entry between screened and control group cancer cases to estimate benefit, then using this estimate plus the difference in survival from time of diagnosis to estimate lead time. Future work involves the use of this approach to derive an estimate of length bias, i.e., the effect of the difference in average sojourn times between screen detected versus interval cases in the screened group.

Evaluating Uncertainty and Sensitivity to Model Assumptions in Parameter Estimates of Bias in Randomized Screening Trials

Randomized screening trials provide information on lead time gained by the screening test (time by which diagnosis is advanced) and benefit (defined either as a reduction in overall mortality or as the time by which survival is extended by virtue of the early detection by screening). Several methods have been suggested to estimate average lead time and average benefit for the population which is offered screening. When applied to data from actual screening trials, the performance of these methods is uncertain, since the true average lead time and average benefit time are unknown. Moreover, the derivation of estimators of such quantities often relies on model assumptions such as screening frequency, test sensitivity, and the distributions of the preclinical and clinical stages of the disease. A computer program has been developed to simulate a randomized trial for purposes of evaluating a given estimator in terms of its accuracy, precision, and robustness to model assumptions. The program allows flexibility in the parameterization of an actual trial, including the distribution of preclinical disease, test sensitivity, and possibly correlation between the estimate of lead time and the estimate of benefit time. Various simulation parameter choices are being investigated to derive information on their effect on proposed estimators of both average lead time and average benefit time.

Geographic Analysis of Cancer Mortality Rates: the Need for Adjustment

If screening is implemented for a particular cancer, in which areas of the country shall we target screening efforts? One answer is to choose counties whose disease rates are high (possibly in relation to their standard errors). However, the 100 such counties that exhibit high rates for a particular disease may be randomly scattered throughout the country; thus it may be difficult to coordinate screening efforts. A better choice would be larger areas comprising several counties, all of whose rates are relatively high. Moreover, the identification of such areas may indicate effects which have heretofore gone unnoticed, due to the presence of one or more very strong risk factors which mask the more subtle risk factors.

Exploratory analysis of cancer mortality rates, specifically for cancers of the lung, prostate, and skin (melanoma), is currently underway to identify such areas. These three cancers represent sites which require adjustment to the rates for some overwhelmingly dominant risk factor: for lung, adjustment for urbanicity (which serves to encompass various environmental effects and is highly correlated with average regional smoking behavior); for prostate, percentage of ethnic composition in the population; for melanoma, latitude. Once rates have been adjusted for these known prognostic indicators, more subtle effects can be deciphered through mapping, effects which may lead to suggestions for types of intervention strategies as well as prime locations for introducing programs of prevention and control.

Two-dimensional Smoothing of Cancer Mortality Rates in U.S. Counties

An exploratory analysis of geographically defined data such as county rates of cancer mortality, possibly adjusted for highly influential factors, involves smoothing the data in two dimensions. Such smoothing needs to be flexible to account for special features of the data, for example, accumulated values (a given data point is actually an integrated summary over an entire region) of nonhomogeneous variance which correspond to unevenly distributed regions of widely varying sizes and shapes. The smoothing also needs to be nonparametric, since standard model assumptions on such data are often violated. A linear smoother for data in the form of standardized rates (e.g., adjusted for age) has been applied to prostate cancer mortality rates, resulting in the identification of interesting features that might otherwise have remained obscured. Open questions remain concerning inference of apparent trends so detected.

Statistical Methodological Research on Two-Dimensional Smoothers

The random variation in data encourages the use of statistical methods to reduce the variation in order to better elucidate underlying patterns. For data that are a function of only one variable, such as number of observed polyps to be modeled by a function of number of fiber servings daily, it is useful to have methods of smoothing the data to reduce the inherent noise in the functional relationship. When data are to be modeled as a function of two variables, such as county mortality rate by geographic location, smoothing is even more important for deciphering patterns in two dimensions. Several two dimensional smoothers are evaluated for their success in capturing underlying patterns, and proposals for other smoothers are being investigated.

BIOMETRY BRANCH STAFF

Office of the Chief

Acting Branch Chief	Laurence S. Freedman, M.A.
Senior Research Investigator	David L. Levin, M.D., M.S.
Cancer Prevention Fellow	Karen Kafadar, Ph.D.
Secretary	Lois Schwartz

Biostatistical Methodology and Cancer Control Epidemiology Section

Section Chief	Charles C. Brown, Ph.D.
Health Statistician	Frances B. Locke, M.S.
Mathematical Statistician	Blossom H. Patterson, M.A.
Visiting Scientist	Victor Kipnis, Ph.D.
Visiting Fellow	Gang Chen, Ph.D.

Clinical and Diagnostic Trials Section

Section Chief	Sylvan B. Green, M.D.
Computer Systems Analyst	Donald K. Corle, M.S.
Mathematical Statistician	Barry I. Graubard, Ph.D.
Biostatistician (IPA)	Paul Smith, Ph.D.
Guest Researcher	Maria Grazia Valsecchi, Ph.D.

Screening Section

Section Chief	Philip C. Prorok, Ph.D.
Mathematical Statistician	Stuart G. Baker, Sc.D.
Mathematical Statistician	Robert J. Connor, D.Eng.
Mathematical Statistician	Richard M. Fagerstrom, Jr., Ph.D.
Cancer Prevention Fellow	Barbara L. Wells, Ph.D.

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CANCER PREVENTION STUDIES BRANCH

OBJECTIVES

The overall objectives of the Cancer Prevention Studies Branch (CPSB) are to identify, develop, and test hypotheses relevant to cancer prevention.

OVERVIEW

The CPSB conducts intramural research in the areas of diet, nutrition and cancer, genetics and cancer, cancer chemoprevention, and other cancer prevention strategies aimed at lowering human cancer risk. This specifically involves:

- Conducting epidemiologic studies relating dietary, genetic, and lifestyle factors to the etiology of cancer;
- Conducting clinical studies of the metabolic effects of dietary changes in humans; and determining the safety, toxicity, pharmacokinetics, bioavailability, and mechanisms of action of macro- and micronutrients;
- Conducting intervention trials to test the effect of nutritional and chemopreventive agents and diet modification in reducing cancer risk;
- Conducting studies to evaluate potential methods of early cancer detection;
- Conducting applied research in the areas of statistical and epidemiologic methods.

ACCOMPLISHMENTS

The Branch has initiated a number of intramural projects in 4 broad areas, including etiologic studies, clinical nutrition studies, prevention trials, and studies of early detection of cancer. These projects represent collaborative efforts in investigating dietary, nutritional, and constitutional factors as well as early detection strategies relating to cancer prevention. The following is a brief summary of the major intramural projects that were active during FY93.

Etiologic Studies:

NHANES I Epidemiologic Followup Survey: Chemoprevention/Nutrition Aspects
(Z01 CN 00104-11 CPSB)

The purpose of the NHANES (National Health and Nutrition Examination Survey) Epidemiologic Followup Study (NHEFS) was to investigate prospectively morbidity and mortality outcomes among the 14,407 adults originally examined in 1971-75. The specific objective of this intramural project was to investigate a number of nutrition and cancer and cancer chemoprevention hypotheses in the NHEFS.

The NHEFS is a prospective cohort study created through a systematic followup of persons examined in the First National Health and Nutrition Examination Survey (NHANES I). NHANES I intended to investigate the health and nutritional status of the United States population, and was particularly targeted toward those population groups hypothesized to be at greatest risk of poor health and nutritional deficiency. The survey was carried out on a probability

sample of the civilian noninstitutional population of the United States from 1971-75. NHANES I included a sociodemographic and medical history, a standardized medical examination, a dietary questionnaire (24-hour recall, with a crude 18-question food frequency questionnaire), hematological and biochemical tests, and anthropometry.

A total of 14,407 men and women aged 25-74 were eligible for inclusion in the NHEFS cohort. Subjects were first traced and interviewed again for the NHEFS in 1981-84. Approximately 93% of the subjects were successfully traced and interviewed. The followup consisted of personal interviews with the subjects or proxies, weight and blood pressure measurements, and the acquisition of hospital and nursing home records and death certificates. The 1981-84 followup originated as a joint project between the National Center for Health Statistics and the National Institute on Aging. Subsequently, several institutes at NIH provided financial support, including the NCI's DCPC and DCE.

Results from NHEFS studies that have been published or in press include:

Dietary fat was not (or, for some analyses, was even slightly inversely) associated with breast cancer. This finding was derived from the 24-hour recall data and was based on 99 cases of breast cancer.

Moderate alcohol consumption (3 or more drinks per week) was positively associated with breast cancer. The association was stronger in younger, leaner, and premenopausal women. No data were available on beverage type or age of drinking.

Serum total cholesterol was inversely related to both cancer incidence and mortality in men and women, with the inverse relation being largely confined to smoking-related cancers. This inverse relation persisted six or more years after cholesterol determination, suggesting that the preclinical cancer hypothesis could not account fully for these findings.

Men and women in the lowest quartile of body stature were at reduced risk of cancer, relative to those in the upper three quartiles. This association was present especially for cancers of the large bowel in men and women, and breast in women.

In a study of self-reported physical activity and cancer, the risk of cancer was elevated in men and women who were very inactive compared to those who were active. Those sites demonstrating the strongest inactivity-cancer relations were large bowel and lung in men, and breast and cervix in women.

Mean total iron-binding capacity was significantly lower and transferrin saturation was higher among men who remained free of cancer. Similar but weaker (and nonsignificant) relations were found for women. Serum albumin was also found to be inversely related to cancer in both men and women.

An excess risk of breast cancer was observed in relation to both stature and frame size in the NHEFS women. Body size defined by weight, relative weight or skin fold measurements was not associated with an increased risk of breast cancer.

The relative risks for standard breast cancer reproductive risk factors were in general agreement with those observed in other studies. Family history and higher education were also found to be associated with an increased breast cancer risk.

In an investigation of a hypothesized association between constipation and breast cancer, breast cancer risk was found to be slightly increased in women with decreased frequency of bowel movements and firm stool consistency.

A small inverse relation was observed between both education and income and all-sites cancer in men and women. This inverse relation largely disappeared when adjustments were made for cigarette smoking.

Adult weight gain in women, as reflected in answers to questions on the lowest and highest adult weights, was found to be positively associated with breast cancer.

Serum retinol was inversely related to the risk of prostate cancer among men in the NHEFS cohort.

Dietary diversity, determined from the 24-hour dietary recalls, was found to be inversely related to mortality in both men and women in this cohort.

A recent analysis suggested that without conscious effort to reduce fat intake, an increase in fruit and vegetable intake *per se* might have only a relatively minor impact on reduction of dietary fat.

Followup of this cohort has continued. The 1986 followup (second wave) of the elderly focused on the 3,850 subjects from NHANES I who were over 75 at the time of the 1981-84 followup. The 1987 followup (third wave) was directed at all 12,385 members of the cohort who were still alive regardless of age. Interviews were completed in approximately 91% of subjects. A fourth wave of followup started in 1991 is nearing completion of the interview phase with editing expected to be complete by early 1995. This followup will extend the period of observation of this cohort to nearly 20 years.

The yield of cancer cases has increased by approximately 40% through the end of the 1987 followup, relative to the number of cases identified through 1984. The additional cases will enable us to examine the relation between alcohol consumption at various ages and subsequent breast cancer risk, since the 1981-84 followup interview ascertained information on drinking during 10-year age periods over a woman's life. Moreover, the food frequency questionnaire administered in 1981-84 can now be used in prospective fashion for subsequent cases so that the relation of, say, dietary fat to various cancers can be analyzed. Other analyses in progress include the modifying effects of physical activity (as a proxy for underlying disease) on the cholesterol-cancer association and the relation of dietary diversity to mortality.

This epidemiologic followup study is conducted as a group effort by several of the National Institutes of Health in collaboration with the National Center for Health Statistics.

Continued Followup of the Breast Cancer Detection and Demonstration Project
(Z01 CN 00143-09 CPSB)

The Breast Cancer Detection and Demonstration Project (BCDDP) screening program began in 1973 in 29 centers in 27 widely dispersed geographic areas of the United States. Initial screening was completed on over 280,000 women over a 2-year period. From the original 280,000 participants in the screening phase of the BCDDP, approximately 64,000 were selected for 5 years of long-term followup (LTF) beginning in 1978, to assess the biology and natural history of breast disease, and to test hypotheses relating to detection, etiology, and survival. Those selected for LTF included all breast cancer cases found during the screening phase, all benign breast disease cases, all those recommended for biopsy, and a sample of "normals." The LTF database will facilitate the exploration of important questions regarding the etiology and natural history of breast cancer. The size of the subcohorts and breadth of data available on them makes this population unique. The large number of cases of both breast cancer and benign breast disease with histologic information available should allow particularly useful analyses of several risk factors in relation to these conditions.

The first 5 years of LTF was completed in September 1986 in all centers. Our analysis of data from the first 5 years of followup found that among women with biopsy-proven benign breast disease there was a direct relation between breast cancer risk and degree of epithelial atypia. Our collaborative analysis of the case-control study of breast cancer among BCDDP participants conducted by the Division of Cancer Etiology has confirmed the direct relation between height and breast cancer risk, and has found an increased risk associated with excess weight among older and/or postmenopausal women.

A mailed questionnaire was sent in 1988-89 to approximately 60,000 subjects traced alive as of 1987. Over 51,000 women responded to this questionnaire, which assessed diet, tobacco and alcohol use, use of menopausal estrogens and progestins, and body weight distribution at various ages. Breast, colorectal, and other cancer cases are currently being identified among these women who completed the 1988-89 questionnaire.

Editing of baseline data from the 1988-89 questionnaire administered to women in the BCDDP cohort, particularly those relating to diet, physical activity, and body size, is currently under way. With the completion of the cancer endpoint assessment in 1993-94, analyses will be carried out on such questions as the relation of dietary fat intake and early life alcohol consumption to breast cancer and the association of dietary factors with colorectal cancer and polyps.

This study is being conducted collaboratively with the Environmental Epidemiology Branch of the Division of Cancer Etiology.

Nutritional Factors and Cancer in the Framingham Heart Study (Z01 CN 00146-05 CPSB)

The objective of this project was to develop a cancer database within the Framingham Heart Study data set in order to carry out etiologic studies of nutrition-related factors and cancer.

The Framingham Heart Study was begun in 1948 to investigate risk factors for cardiovascular disease. The original cohort consisted of 5,209 men and women aged 30-62 at baseline who received biennial examinations consisting of medical histories, physical examinations, and a variety of laboratory tests.

The CPSB collaborated with Boston University and the National Heart, Lung, and Blood Institute (NHLBI) investigators to develop a Framingham Cancer file consisting of all incident malignancies developing during the lifetime of each of the cohort members. Over 1,000 malignancies have been identified in this cohort, including over 150 breast cancers in women and nearly 200 large bowel cancers in men and women combined.

The initial impetus for investigating cancer as an endpoint in the Framingham Study was the alcohol-breast cancer hypothesis. Five epidemiologic cohort studies and a majority of case-control studies had demonstrated a positive association between moderate alcohol consumption and breast cancer, with relative risks in the range of 1.5-2.0. Framingham provided an opportunity to investigate this finding in another cohort study.

No association between alcohol consumption and breast cancer was observed in this cohort. It was not possible to exclude an excess breast cancer risk among women consuming more than one drink per day. It has been noted that the Framingham women were in their teenage years or 20's during Prohibition in the U.S. If alcohol were to affect breast carcinogenesis only when consumed during early life, then it is plausible that recent/later life consumption is a poorer proxy for early life consumption in the Framingham study than in other cohorts.

The availability of cancer data for this cohort has made it possible to examine the relation between other nutrition-related factors and cancer. We have shown positive associations between body fat distribution (central adiposity ratio, defined as the ratio of the sum of

central/peripheral skinfold thicknesses) and breast cancer in women. There was no association between degree of adiposity, as measured by the sum of the five skinfolds or by body mass index (weight in kg divided by height in m^2) and subsequent breast cancer.

We also studied the relation between self-reported physical activity and large bowel cancer. Inactivity was associated with an increased risk of large bowel cancer in men but not women. The narrow range of physical activity and the minimal heavy activity reported by women in this cohort may have limited our ability to detect an inverse association in women.

We have recently completed an analysis of serum lipoproteins and large bowel cancer. This study is an update, with specific lipoprotein fraction data, of earlier work in Framingham which showed an inverse cholesterol-cancer relation that persisted even several years or more after cholesterol measurement. We are also investigating the relation of physical activity and breast cancer in women; this required a detailed review of cohort members' records to provide a more precise estimate of physical activity. A study of serum HDL in relation to breast cancer is also under way.

We will be working with collaborators this next year in updating the cancer files for this study. This will permit prospective analyses of, for example, alcohol and breast cancer in the Offspring cohort and more detailed analyses of physical activity and alcohol consumption in relation to breast and large bowel cancer in the original cohort.

This study is being conducted collaboratively with investigators from Boston University in Boston, MA and the National Heart, Lung and Blood Institute.

Nutritional Factors and Cancer in the Framingham Offspring Study
(Z01 CN 00147-05 CPSB)

The objective of this project was to develop a cancer database within the Framingham Offspring Study data set in order to carry out etiologic studies of nutrition-related factors and cancer.

The Framingham Offspring Study, begun in 1971, comprised 5,135 children (2,489 men, 2,646 women) of the original Framingham Heart Study cohort. The Cycle 1 (baseline) examination was carried out from 1971-77; the Cycle 2 and 3 examinations were conducted, respectively, in 1979-82 and 1984-85. The Cycle 4 examination is ongoing.

Interest in the alcohol-breast cancer hypothesis was again the primary rationale for collaborating with Boston University and NHLBI investigators in developing a cancer file in this cohort.

Two hundred forty-six (246) cancers, including 43 breast cancers in women, have been identified through Cycle 3. Case identification based on information from Cycle 4 is ongoing; this Cycle is expected to be completed over the next one to two years.

In addition to analyzing alcohol and breast cancer, it will be possible to examine the cholesterol-cancer relation in this cohort. Data on body size, diet (an intensive dietary history was administered at Cycle 3), physical exercise, and serum hormones (estrogen and testosterone laboratory analyses are completed) will also be available for future analysis.

As with the original Framingham Heart Study, we expect to be updating the cancer files for this study in the coming year.

This study is being conducted collaboratively with investigators from Boston University in Boston, MA and the National Heart, Lung, and Blood Institute.

Finland Studies of Nutrition and Cancer (Z01 CN 00148-05 CPSB)

The important relationship of diet and nutrition in the development of cancer has become well known through various research efforts. Laboratory studies have shown cancer inhibitory function for various natural and synthetic nutrients in various models, which have been corroborated by human epidemiologic studies of nutrient intake, tissue levels, and cancer incidence.

The objectives of these etiologic studies are to 1) assess the role of fats; selenium; and vitamins A, E, and C in breast cancer development; and 2) evaluate the relation of intake of various nutrients to subsequent cancer, particularly breast, colon, and lung.

The project includes two studies. The first is a breast cancer case-control study of fats; total calories; selenium; and vitamins A, E, and C. The role of various anthropometric measurements, genetic markers for breast cancer and reproductive factors are also being explored. To date, basic hormonal and reproductive risk factors have been analyzed. Results support several of the established risk associations, and specifically identify total lifetime duration of menstrual activity as a determinant of breast cancer risk.

The second project is a comparison of nutrient intakes in cases and reference subjects identified from an existing large cohort with prediagnostic baseline dietary histories. Associations between various dietary components and several cancers, including breast and lung, are being assessed. Analyses to date have revealed an inverse association between energy intake and breast cancer risk, and a significant positive association for energy-adjusted total fat intake and breast cancer. The fat association was more strongly associated with intake of mono- and polyunsaturated fats than saturated fats. The anti-oxidant vitamins C and E and carotenoids reduced lung cancer risk, but primarily among nonsmokers. Fruit intake was also shown to be protective.

These studies are being conducted collaboratively with the DCPC Surveillance Program and the National Public Health Institute and Social Insurance Institute of Finland.

Yunnan Tin Miners Lung Cancer Studies (Z01 CN 00149-05 CPSB)

As part of our general collaborative studies in China and the feasibility study for a lung cancer intervention study among Yunnan tin miners, two lung cancer case-control studies were conducted among tin miners employed by the Yunnan Tin Corporation (YTC). The first, a prevalence case-control study, interviewed 107 living cases diagnosed between 1967-84 and an equal number of matched controls. A second study includes 183 lung cancer cases incident in 1985 and 1986 among miners and an equal number of matched controls. Data concerning smoking, occupational exposures including radon and arsenic exposure, diet, and other exposures were collected by personal interview. Analysis of the case-control data on tobacco and radon exposure among the initial 107 cases indicated that there was a modest increase in risk with smoking, primarily for water pipe use; workers in the highest quarter of radon exposure had a 10-fold increase in risk compared to nonexposed workers; higher risk was associated with long duration as opposed to high rate of exposure; and risk was greater for radon as opposed to tobacco exposure. Analysis of the arsenic exposure data in these cases and controls found a strong linear dose response relation with a risk in excess of 20-fold for the highest quarter of arsenic exposure. When compared with tobacco, arsenic exposure was the greater risk in these data. Workers whose sole arsenic exposure came from underground mining had nearly the same risk of lung cancer as did those with exclusively smelter exposure. Detailed quantitative modeling of a subset of these data found no modification of risk among workers with early age at first exposure. Analyses of risk by diet in the incident case-control study found that even after controlling for other risk factors (i.e., radon, arsenic, tobacco), a protective effect was observed among men with the highest (as compared with the lowest) intakes of yellow/light green vegetables and tomatoes.

A new study among the tin miners at the YTC was initiated in 1992 to establish a biologic specimen bank for the study of early markers of lung cancer. A secondary aim of that study is the establishment of a cohort for the study of environmental (including dietary) and genetic risk factors for lung cancer. See Z01 CN 00176-02 CPSB for further description.

These studies are being conducted collaboratively with scientists from the Labor Protection Institute of the Yunnan Tin Corporation, the Johns Hopkins University, the Cancer Institute of the Chinese Academy of Medical Sciences, and the Division of Cancer Etiology at the NCI.

Esophageal Cancer Genetics Studies (Z01 CN 00150-05 CPSB)

The overall goal of this project is to develop an understanding of the genetic as well as environmental influences involved in the etiology of esophageal cancer. The study is being conducted in North Central China where rates of this cancer are the highest in the world and where genetic factors are suspected of playing an important role. Specific objectives of the studies are 1) to determine if esophageal cancer aggregates in families, 2) to study the genetic transmission or segregation of esophageal cancer within families, 3) to distinguish genetic versus environmental influences in the etiology of esophageal cancer, and 4) to assess family history of esophageal cancer as a risk factor for the disease. To accomplish these objectives, data from several sources have been drawn together, including multi-generation family pedigrees collected in 1979 from an entire county in Shanxi Province, supplemented with information from a subset of families in selected villages who were reinterviewed in 1989 to update pedigrees and vital status data; multi-generation family pedigrees from 24 high-risk families in Henan Province; and a small case-control study conducted in Shanxi Province.

Results from the small case-control study indicate that subjects with a positive family history had nearly an 8-fold risk of esophageal cancer compared to subjects with a negative family history. From the households interviewed in 1979 and followed up in 1989, more families with prior esophageal cancer history reported new esophageal cancer deaths during the ten year followup period than families without prior history (19% versus 5%), providing strong evidence for familial aggregation. Segregation analysis using data from Henan Province were most compatible with genetic transmission by an autosomal recessive gene. Further analyses in progress include examination of the esophageal cancer risk among first degree relatives of cases and segregation analysis of data from Shanxi Province.

This study is being conducted collaboratively with scientists at the Chinese Academy of Medical Sciences and the Fox Chase Cancer Center in Philadelphia, PA.

Fels Early Nutrition and Growth Study (Z01 CN 00154-04 CPSB)

This project is designed to investigate the relation of childhood nutrition to breast cancer risk factors, including age at menarche, adult height, weight, and fatness. Secondary purposes include tracking the development of overweight and obesity from birth through young adulthood, identification of possible "sensitive" or high-risk periods (with respect to obesity) in childhood, and—more important—to identify the contribution of diet to the development of childhood and adult obesity.

Detailed anthropometric data (height, weight, skinfold thickness, etc.) and demographic characteristics available from the Fels Study and the Division of Human Biology of the Wright State School of Medicine have been linked to calorie, macro- and micronutrient data for 106 girls. Adult height and weight are also available. Nutrients include the following: total energy (kilocalories); total fat, protein, and carbohydrate; saturated, polyunsaturated, and monounsaturated fat; cholesterol; dietary fiber; and vitamins and minerals (from food and supplementary sources).

A validation substudy of remote dietary recall is also being conducted. Retrospective food frequency questionnaires asking about food habits during adolescence are being sent to some of the now adult women. These will be compared to dietary records for these females as teenagers.

This study is being conducted collaboratively with scientists at the Wright State School of Medicine in Yellow Springs, OH.

Clinical Nutrition Studies:

Human Studies of Diet and Nutrition (Z01 CN 00101-11 CPSB)

The role of dietary factors in cancer prevention has been assessed in animal experiments, in human epidemiologic studies, and most recently, in prevention trials. For many of these agents, however, information is incomplete concerning their safety, toxicity, dose, form, bioavailability, pharmacokinetics, and mechanisms of action. To further define these parameters in humans, a cooperative research effort between the Beltsville Human Nutrition Research Center (BHNRC), U.S. Department of Agriculture, and the CPSB is being conducted. Initial efforts in this collaboration focused on three nutrients that have shown the most promise for cancer prevention: selenium, fat, and beta-carotene. More recently, studies have examined alcohol, omega-3 fatty acids, and vitamin C.

Selenium Studies

A study examining a single, oral dose of two forms of stable labeled selenium (as selenite and selenomethionine) in the fasting and nonfasting state was conducted to investigate the pharmacokinetics of selenium. Two distinct, complex, multicompartmental models have been developed to explain the kinetics of selenite and selenomethionine.

To evaluate potential toxicity from long term ingestion of high levels of selenium, interviews, physical examinations, biologic samples, and duplicate meals have been collected for selenium analysis from 142 subjects residing in South Dakota and Wyoming where soil levels (and consequent blood levels) of selenium are the highest found in the U.S. In spite of the high selenium intake and serum levels in subjects from these areas, physical findings characteristic of selenium toxicity were not seen, and no association was observed between the various indices of selenium status and frequency of self-reported symptoms, hematological or biochemical parameters, or abnormalities seen on photographs of nails. The relation of selenium status to age, gender, and current smoking has also been evaluated. Men and women had similar mean values of serum, whole blood and toenail selenium despite higher intake among men. Smokers had lower tissue selenium levels than did nonsmokers due, at least in part, to lower selenium intake. Age was not associated with tissue selenium content in these subjects. Work is in progress to evaluate estimations of selenium intake from selenium concentration in serum, whole blood, urine, and toenails.

Data from the South Dakota and Wyoming subjects have also been used to examine the reproducibility and validity of the Willett food frequency questionnaire. Previous validation studies of this questionnaire have all been done in populations capable of reporting their diet with unusual accuracy (i.e., nurses, health professionals, etc.). Results of this analysis indicate that the validity in this population was similar to previous groups. This finding supports the use of this or similar dietary assessment instruments in studies of general populations.

Fat Studies

Studies examining the metabolic effects of changes in dietary fat and fiber have been conducted separately in premenopausal women, men, and postmenopausal women.

Premenopausal Women's Dietary Fat Study: The first study of fat examined the metabolic effects of 40% versus 20% of calories from fat in premenopausal women eating controlled diets at two different ratios of polyunsaturated to saturated fats (P:S) for eight menstrual cycles. Study results to date have shown that the low-fat diet was associated with an insignificant reduction in serum cholesterol and a significant increase in serum triglycerides; alterations in lipids measured in exfoliated cheek cells; a lengthening of menstrual cycle when assessed by self-report or biochemically with LH measurements; a reduction in the number of insulin receptors in erythrocyte ghosts; lower plasma levels of DHEA-S and cortisol and higher levels of plasma insulin; P:S-ratio-specific changes in bile acid levels; no change in the level of fecapentaene, a potent fecal mutagen; cycle-phase and fat-level specific alterations in lipoprotein and red blood cell fluidity; and alterations in body composition as indicated by a reduction in percent body fat. Menstrual cycle effects on plasma lipids and certain hormones were also observed.

Sex hormone analyses were recently completed and indicate that the low-fat diet was associated with significant decreases in plasma total and SHBG-bound estradiol during both the periovulatory and luteal phase samples for women on the P:S=0.3 diet. No significant changes in women on the P:S=1.0 diet were seen for any of the hormones measured. When the two diet groups were combined, plasma total and albumin-bound estradiol levels on the low-fat diet were significantly lower in the luteal phase, while estrone sulfate was significantly lower in the follicular phase. These changes are consistent with the hypothesis that lowered dietary fat reduces the risk of breast cancer through changes in sex hormones and that type of fat consumed may also be important.

Men's Dietary Fat Study: The second study of metabolic parameters associated with fat intake was conducted in healthy men on a controlled high-fat, low-fiber diet. The parameters were compared to measurements on samples collected from the same subjects while on a controlled low-fat, high-fiber diet. Results of lipid determinations indicate that total cholesterol, LDL-cholesterol, and HDL-cholesterol were 17-20% lower in men on the experimental as compared to the reference diet. The percent reductions in lipids were similar whether the men initially had total cholesterol levels of 200 or more or less than 200, suggesting that the cholesterol-lowering effect of the low-fat, high-fiber diet is not confined to men with markedly elevated cholesterol levels. Cholesterol was lowered on the experimental diet in all but one of the subjects, raising the possibility that cholesterol could be a valuable adherence marker in intervention studies involving a combined low-fat, high-fiber diet. The experimental diet, with 6.6% of calories from polyunsaturated fat, was associated with a 14% reduction in prostaglandin synthesis as measured by the urinary excretion of 7-alpha-hydroxy-5,11-dioxo-tetranorprostaten-1,16-dioic acid. These results support the hypothesis that dietary lipid changes can substantially alter the *in vivo* production of E-series prostaglandins.

Results from other aspects of this study can be summarized as follows. Lipid phase fluidity, determined by DPH fluorescence polarization, increased significantly in VLDL, LDL, and HDL on the experimental, as opposed to the reference, diet. Calcium, magnesium, manganese, iron, zinc, and copper intake, and fecal excretion were significantly higher on the experimental compared to the reference diet. Moreover, calcium, magnesium, zinc, and copper showed significantly higher apparent retention on the experimental diet, suggesting that a low-fat, high-fiber diet containing mineral levels above the recommended dietary allowance can result in positive mineral balance. A validation study that involved the administration of several physical activity questionnaires and the measurement of resting energy expenditure indicated that the estimates of individual energy expenditure was suboptimal for the questionnaires, but the questionnaires did provide reasonable group means for the various physical activity parameters. Results from analyses of fecal mutagenicity (especially the SOS test), hormones, bile acids, and cheek cell fatty acids (a potential marker of qualitative dietary fat intake) are still pending. Preliminary analyses indicate a small reduction in serum testosterone and more substantial significant reductions in urinary estradiol and estrone on the experimental as opposed to the reference diet.

Postmenopausal Women's Dietary Fat Study: The third study of fat examined primarily lipid and hormone measures in postmenopausal women, contrasting their free-living uncontrolled diet values with those on a controlled, low-fat (20 percent of calories) diet. Preliminary analyses have not shown significant differences in the lipid and hormone levels examined.

Carotenoid Studies

Two human studies have been conducted examining the plasma response to ingestion of selected carotenoids in various forms, including supplements and selected foods high in carotenoids. The first study involved a single ingestion while the second involved daily prescribed amounts given as part of a controlled diet over a six-week period.

Results from the single ingestion study indicate that the efficiency of carotenoid absorption varied widely (3 to 4-fold) in normal subjects, that peak plasma beta-carotene response from capsules occurred in 24 to 48 hours, that a large dose of carrots produced a small but measurable increase in plasma beta-carotene while no response was seen with broccoli or tomato juice, and that plasma response to pure beta-carotene was substantially greater (approximately 6-fold) than response to a similar amount of beta-carotene from carrots.

In the chronic intake study, plasma beta-carotene increased in subjects given beta-carotene capsules or fed carrots, alpha-carotene increased in subjects fed carrots, lutein increased in subjects fed broccoli, and no changes in plasma carotenoids were seen in subjects fed tomato juice. Subjects given purified beta-carotene in capsules exhibited greater average maximum change in plasma levels by a factor of over 5-fold than did subjects fed similar quantities of carotenoids from food sources alone. An additional observation from this study was that carotenodermia (yellowing of the skin) was observed in all five subjects who took 30 mg but in none of the five who took only 12 mg of purified beta-carotene daily.

A study of healthy premenopausal women was conducted this year to examine the distribution of individual carotenoids on lipoprotein fractions and to assess the fluctuation in plasma carotenoid and lipoprotein cholesterol levels by phase of the menstrual cycle. The study included evaluation of the diet-plasma carotenoid relation during both a 1-month free-living period as well as during a 2-month controlled feeding study. During the controlled diet period, women were fed a 7-day menu cycle with a standard daily amount of carotenoid-rich foods in order to keep the three major carotenoids (beta-carotene, lutein, and lycopene) at constant levels of intake. Blood is being analyzed for individual carotenoids and the distribution of the carotenoids on the lipoprotein fractions, and plasma lipids and hormones (estradiol, luteinizing hormone, progesterone) are measured during specific phases of the menstrual cycle. Results from this study should enhance our understanding of the etiologic pathway from diet to cancer risk in women and provide data upon which to base timing of blood specimen collections in future cancer etiology studies in premenopausal women.

Cross-sectional data from two other previously conducted NCI-USDA human studies were analyzed to examine the diet-plasma carotenoid relation. In both studies, dietary carotenoid intake data were available from seven days of food records as well as a food frequency questionnaire. The data from both sets of dietary tools were linked to the USDA-NCI carotenoid food composition data base. Among both men and women, a significant diet-plasma carotenoid relation was seen using both the food records and the food frequency questionnaires, but the magnitude of the relation varied by individual carotenoid. The correlations were generally higher among the women then the men, which may be attributed to the use of newly developed HPLC methodology for the plasma analysis. These findings have implications for use of food frequency questionnaires to examine the carotenoid-cancer relation in larger epidemiologic studies where food frequency questionnaires are generally used.

Smoking, alcohol, and other lifestyle factors are known to influence plasma carotenoid levels. It is unknown, however, whether these influences are due to differences in dietary intake or to metabolic changes. Using extant data from the alcohol study (see below), the potential metabolic effect of alcohol on plasma carotenoid levels is being examined in women who participated in a controlled feeding study in which carotenoid intake was fixed and alcohol was varied.

A carotenoid food composition data base with values for the five major carotenoids in humans has been developed for over 2,300 foods in the U.S. diet. This data base plus documentation are currently available for use by the scientific community. Two publications, which are part of the documentation, describe the development and application of the carotenoid food composition data base.

Alcohol Study

The potential role of alcohol consumption in the etiology of breast cancer has been prominent in several recent studies and is particularly important because it is a risk factor that can be modified. While this hypothesis requires verification in other epidemiologic studies, we conducted a clinical metabolic study to examine the effect of alcohol ingestion on hormonal status as one potential mechanism of action. Results from the controlled feeding component of the study indicate that consumption of the equivalent of two drinks per day in 34 premenopausal women resulted in significant elevations in several hormones. Significant increases were seen in plasma DHEA sulfate in the follicular phase, while in the periovulatory phase plasma estrone and estradiol and urinary estradiol were increased; in the luteal phase urinary estriol, estradiol, and estrone were all increased. Although no changes were found in the proportion of bioavailable estradiol, the increased total estradiol in the periovulatory phase implies increased absolute amounts of bioavailable estradiol. These results suggest that alcohol consumption may lead to greater exposure of the breast to total and bioavailable estrogens. Analyses of the relation of amount of alcohol use to hormone levels in the cross-sectional component of the study, however, have not confirmed these findings.

Omega-3 Fatty Acid Study

A number of animal and human studies have suggested a protective role for omega-3 polyunsaturated fatty acids in carcinogenesis. In order to understand more clearly the underlying mechanisms of this role, we initiated a controlled feeding study in which a number of metabolic parameters most likely to be affected by feeding omega-3 fatty acids from fish oils are evaluated. Primary among these parameters are effects on prostaglandin biosynthesis, immune function, and prooxidant stress. Forty healthy male volunteers 24-57 years of age consumed a controlled basal diet providing 40% of energy from fat (P:S ratio about 0.8:1), 130 mg/1000 kcal cholesterol, and a minimum of 23 mg/day of alpha-tocopherol for three experimental periods lasting a total of 28 weeks. During period 1 (10 weeks) the diet was supplemented with placebo capsules (15 x 1 g/day) consisting of a blend of fats approaching the fatty acid profile of the basal diet. This was followed by a second 10-week period during which the subjects received 15 x 1 g/day capsules of fish oil concentrate. During period 3 (8 weeks) they continued the 15 g/day intake of fish oil concentrate but received an additional 200 mg/day of alpha-tocopherol.

Results to date have shown that fish oil supplementation resulted in reductions in prostaglandin M (the excretory metabolite of prostaglandin E), 11-dehydrothromboxane B2 (the primary urinary metabolite of thromboxane), and PGI_2-M (the major urinary catabolite of prostacyclin); suppressed blastogenesis as measured by the mitogenic response of peripheral blood mononuclear cells, an effect that was reversed by concurrent supplementation with alpha-tocopherol; decreased plasma insulin, glucagon, and somatomedin-C levels; a decrease in beta-endorphin, an opiate involved in lipid metabolism; and lower membrane cholesterol:phospholipid ratios and increased membrane tocopherols but no change in insulin binding or RBC membrane fluidity, while concurrent supplementation with alpha-tocopherol resulted in increased insulin binding and membrane fluidity.

Vitamin C Study

There have been numerous epidemiologic studies of the relation between cancer and fruits, vegetables, or indices derived from them. The vast majority of studies have found significant protective effect for one or more fruits or vegetables. Despite the clear evidence regarding fruits and vegetables themselves, it cannot be said definitively which constituents of fruits and vegetables are protective. At least part of the difficulty in ascribing protective effect to specific nutrients is due to uncertainty about the nutrient content of foods as actually consumed. Vitamin C is one of the prime candidates among the potentially protective nutrients found in fruits and vegetables. To better understand the bioavailability of vitamin C administered in different forms, including tablets, fruits, and raw and cooked vegetables, a controlled feeding study was conducted.

Results to date indicate that ascorbic acid ingested as equal amounts of cooked broccoli, orange juice or fruit, or in synthetic form appears to equally bioavailable. The bioavailability from raw broccoli was 20% lower than the other ascorbic acid sources, a difference that is unlikely to be of practical importance. Urinary malondialdehyde, one measurement proposed to monitor exposure to oxidative stress, was examined during ascorbic acid supplementation and depletion. Although levels were lower in repletion than during depletion, the putative time of stress, the pattern was somewhat inconsistent. Using diet estimates from a baseline dietary questionnaire, determinants of plasma ascorbic acid were assessed. Higher dietary intake of vitamin C was the major determinant of plasma ascorbic acid. Vitamin C supplement users also, predictably, had higher plasma ascorbic acid levels. Body Mass Index was also a determinant, with lower plasma ascorbate levels found in persons with higher BMIs. An evaluation of factors influencing plasma tocopherol levels showed that dietary vitamin E intake was positively associated with plasma alpha-tocopherol and negatively associated with plasma gamma-tocopherol levels. Plasma lipids, BMI, dietary fat, and alcohol use were also shown to influence tocopherol levels in nonsupplement users.

Evaluation of the Effects of a Fat-Modified Diet on Hormones During Adolescence
(Z01 CN 00153-04 CPSB)

This study is ancillary to the Diet Intervention Study in Children (DISC), sponsored by the Division of Epidemiology and Clinical Applications, National Heart, Lung, and Blood Institute (NHLBI). DISC is a multicenter, randomized clinical trial designed to evaluate the feasibility, safety, and efficacy of a fat modified diet during adolescence to lower LDL-cholesterol. The NCI sponsored ancillary study will evaluate the effect of this fat modified diet on sex hormones during adolescence. The effect of the diet on total concentrations of hormones and bioavailable fractions of hormones will be determined. The NCI sponsored ancillary study will also identify characteristics of adolescents that affect sex hormone levels and bioavailability of sex hormones; these include age, Tanner stage, anthropometric measures, physical activity, and dietary intake.

DISC is being conducted as a cooperative agreement between NHLBI, six clinical centers, and a coordinating center. The first participants were randomized into the feasibility study in the spring of 1988 and recruitment was completed in 1990.

All six DISC clinical centers have agreed to participate in the NCI sponsored ancillary study on hormones. A total of 663 children were randomized into the intervention and control groups. Children enrolled in the trial were girls 7.8-10.1 years old or boys 8.6-10.8 years old who had LDL-cholesterol levels between the 80th and 98th percentile but who were otherwise healthy. Dietary goals for the intervention group are to limit fat intake to 28 percent of calories and increase the ratio of polyunsaturated to saturated fats to approximately 1. Cholesterol intake will be restricted to 75 mg/1000 calories. Children in the control group follow their usual diets.

Currently, DISC is funded through 2000, which will allow followup of all children until age 18.

This study is being conducted collaboratively with scientists from the National Heart, Lung, and Blood Institute in Bethesda, MD; Children's Hospital in New Orleans, LA; the Johns Hopkins University in Baltimore, MD; the Kaiser Center for Health Research in Portland, OR; the Maryland Medical Research Institute in Baltimore, MD; the Medical College of New Jersey in Newark, NJ; Northwestern University in Chicago, IL; the University of Pittsburgh in Pittsburgh, PA; and the University of Iowa in Iowa City, IA.

Prevention Studies:

Alpha-Tocopherol, Beta-Carotene Lung Cancer Prevention Study
(Z01 CN 00100-11 CPSB)

The Alpha-Tocopherol, Beta-Carotene Lung Cancer Prevention Study (ATBC Study) is investigating the efficacy of daily oral alpha-tocopherol (50 mg) and beta-carotene (20 mg) in a double-blind, randomized 2x2 factorial design trial aimed at preventing lung cancer among 50-69 year old male cigarette smokers. The project is based on experimental and epidemiological research that demonstrates a potential preventive role for these agents. Recruitment took place between 1985-88, and the active intervention phase of the trial ended in March of 1993 after an average followup of over 6 years. A postal survey screening for potential trial participants was sent to 291,000 men in southern Finland, and 76% responded. We invited the smokers willing to participate (43,000) to one of 13 study clinics, and over 29,000 were randomized into the study. Compliance to the one capsule daily regimen has remained very high (97% average), and the dropout rate averages less than 6% per year. Reduction of lung cancer incidence in the active agent groups is the primary study goal; differences in the occurrence of other cancers will also be evaluated. Several pilot studies in support of the trial have also been completed including a feasibility study, validation of study dietary questionnaires, and evaluation of skin yellowing and serum levels following beta-carotene administration.

This study is being conducted in Finland because of their traditionally high lung cancer rate, ready access to a high-risk population, and excellent country-wide cancer registration system. This trial is being conducted collaboratively with the Surveillance Program of the Division of Cancer Prevention and Control and the National Public Health Institute of Finland.

An additional study ancillary to the ATBC Study was initiated that will determine the effect of the intervention agents on the development and progression of chronic atrophic gastritis, gastric dysplasia, and gastric adenocarcinoma—conditions with high prevalence in Finland and other parts of the world. This ancillary study is being conducted collaboratively with the Biometry Branch from the Division of Cancer Prevention and Control and investigators in Finland.

Use of Isotretinoin in Prevention of Basal Cell Carcinoma (Z01 CN 00103-11 CPSB)

This study is a 5-year, randomized, double-blind prevention trial designed to evaluate the effectiveness of low dosage levels of isotretinoin in reducing the incidence of basal cell carcinoma (BCC) tumors in a high-risk population and to examine possible side effects associated with long-term administration of low doses of isotretinoin. A total of 981 subjects were entered into the study over a 36-month period at eight participating clinical centers located around the country. At each center, subjects were randomly allocated to intervention (10 mg/day) or control (placebo) groups during the recruitment period which concluded in June 1987.

Vitamin A and its analogs, collectively known as retinoids, have been actively studied for several years in relation to their requirements in normal physiology and health, as well as for their potential in prevention of human disease. This vitamin is necessary for the differentiation of epithelial cells and is essential for the development and function of growth, reproduction, and vision. Deprivation or deficiency of vitamin A promotes tissue metaplasia and neoplasia in various animal and organ culture models. Supplementation with retinoids can reverse these changes and restore functions of cell growth and differentiation in various cell lines.

Laboratory experiments have shown that retinoids administered to animals can prevent chemical carcinogenesis. Since in most of the experiments animals were administered retinoids after their exposure to the carcinogen, the prophylactic effect of the retinoids is believed to be in the post-initiation phase, i.e., during promotion of carcinogenesis. In addition, several epidemiologic studies have shown an association of low dietary intake or serum levels of vitamin A with increased risk of cancer, notably lung cancer and other tumors of epithelial origin. Recent case reports have shown that isotretinoin can prevent the appearance of new BCC tumors for 4 years in patients at high risk of developing new tumors.

The 3-year intervention phase of the study ended on June 30, 1990. All patient followup ended in June 1991 and the study was officially closed in September 1991. Major statistical analyses have been completed and manuscripts reporting the results of the 3-year intervention phase has been published. The study found that after three years of intervention, there was no treatment group difference in either the cumulative incidence of the first new BCC or the annual rate of BCC tumor formation. Elevated serum triglycerides, hyperostotic axial skeletal changes, and mucocutaneous reactions were more frequent in the isotretinoin group ($P<0.001$). We concluded that this low dose regimen of isotretinoin is not effective in reducing the incidence of new BCC tumors in patients with previous BCC and is associated with adverse systemic effects.

This study was conducted collaboratively with the Surveillance Program of the Division of Cancer Prevention and Control; the Walter Reed Army Medical Center in Washington, DC; the Fitzsimmons Army Medical Center in Aurora, CO; the Brooke Army Medical Center in San Antonio, TX; the Eisenhower Army Medical Center in Augusta, GA; the Portsmouth Naval Medical Center in Portsmouth, VA; Northwestern University in Chicago, IL; the University of Arkansas in Little Rock, AK; and the Roswell Park Memorial Institute in Buffalo, NY.

Nutrition Intervention Studies of Esophageal Cancer in Linxian, China
(Z01 CN 00112-10 CPSB)

The purpose of this project is to conduct two intervention trials using multiple vitamin-mineral supplements to evaluate the relation between such supplements and esophageal cancer mortality. These two studies are being conducted in Linxian (Henan Province) in the People's Republic of China (PRC). Linxian, a rural county with population of 800,000, was selected because it has the highest rate of esophageal cancer in the world and because there is suspicion that the population's chronic deficiencies of multiple nutrients may be etiologically involved. The Dysplasia Trial includes 3,318 subjects with cytologic evidence of dysplasia who took intervention agents from May 1985-May 1991 in a two-arm design (multivitamins/minerals versus placebo). The General Population Trial randomized 29,584 individuals from the general population who participated in the intervention from March 1986-May 1991. This trial uses a more complicated fractional factorial design to allow evaluation of four separate factors, including vitamin A + zinc, riboflavin + niacin, vitamin C + molybdenum, and beta-carotene + vitamin E + selenium.

In October 1987, at the midpoint of the Dysplasia Trial, a series of examinations were conducted to evaluate potential endpoints considered to be intermediate in the carcinogenesis process. A repeat balloon cytologic examination was conducted on 2,824 participants and an

endoscopic examination and blood collection were performed on 850. Analyses of samples collected during these examinations have included assessment of esophageal cytology, histology, cell proliferation, and DNA-content as well as measures of immune function and other studies.

In March, April, and May 1991, at the conclusion of intervention for the 6-year Dysplasia Trial and the $5\frac{1}{4}$ year General Population Trial, end-of-trial questionnaires were administered to 24,581 study participants; blood samples were taken from 6,196; cytologic examinations were performed on 5,424; and endoscopic examinations were carried out on 878. Analysis of the data from these surveys is currently underway.

Results from the General Population Trial show significantly lower total mortality, cancer mortality and stomach cancer mortality among participants who received beta-carotene/vitamin E/selenium supplementation. Subjects who received niacin and riboflavin also showed a reduction in esophageal cancer incidence. Results from the Dysplasia Trial were similar but less striking. Modest reductions in total and cancer mortality were seen, but cumulative cancer incidence rates were the same in the supplemented and placebo groups. Post-intervention followup is ongoing for participants from both trials.

This study is being conducted with the Biostatistics Branch of the Division of Cancer Etiology at the NCI in collaboration with the Cancer Institute of the Chinese Academy of Medical Sciences.

A Dietary Intervention Study of the Recurrence of Large Bowel Adenomatous Polyps (Polyp Prevention Trial) (Z01 CN 00151-05 CPSB)

The primary objective of this study is to determine whether a low-fat, high-fiber, and high-fruit and vegetable dietary pattern will decrease the recurrence rate of adenomatous polyps of the large bowel. A secondary objective of this study is to determine how this dietary pattern affects markers of large bowel epithelial cell proliferation, whether the proliferation markers predict neoplasia (polyp recurrence), and to what extent changes in proliferation indices account for the observed intervention effect.

Large bowel cancer is the second leading cause of death from malignant disease in the United States. It is estimated that over 150,000 persons in this country will be diagnosed with large bowel cancer this year and over 60,000 people will die from the disease.

The evidence that diet plays a key role in large bowel carcinogenesis is strong and growing. A large body of ecologic, analytic epidemiologic, human metabolic, and animal experimental data suggests that three dietary factors increase the risk of large bowel cancer: high dietary fat, low dietary fiber, and low fruit and vegetable intake. The intervention diet is targeted toward achieving a dietary pattern consisting of 20% of calories from fat, 18 g of total dietary fiber per 1000 kcal, and 5-8 daily servings of fruits and vegetables. The usual diet for the control group, based on data from the National Health Interview Survey, is expected to comprise 36-38% of calories from fat, 10-15 g per day of dietary fiber, and 3.5 servings of vegetables and fruits daily. By embracing three promising dietary hypotheses simultaneously, the low-fat, high-fiber, vegetable and fruit-enriched intervention diet is intended to maximize the possibility of reducing polyp recurrence. We emphasize that the demonstration of any effect of diet on the neoplastic process would be a major advance that would spur further research in this area.

Fat, fiber, and vegetables and fruits do not represent the only dietary hypotheses for large bowel cancer. However, a dietary pattern comprising low fat, high fiber, and enhanced vegetable and fruit consumption is likely to be accompanied by reductions in the consumption of, for example, meat, food mutagens, and total calories, each of which has been implicated in large bowel carcinogenesis.

Large bowel adenomas (polyps) present a unique opportunity to conduct an intervention trial because of the high prevalence of these lesions in the general population (over 30% in adults over 50 years of age), the high polyp recurrence rate (over 10% annually) in those who have undergone polypectomy and the strong link between polyps and cancer. It is generally accepted that large bowel adenomas are an obligate precursor lesion for most large bowel cancers. An intervention, therefore, that reduces the recurrence of large bowel polyps would have a strong likelihood of reducing the incidence of large bowel cancer.

Micro-level mechanisms in large bowel carcinogenesis (or events tightly linked to such mechanisms) may serve as intermediate endpoints if an exposure-induced change in the endpoint would necessarily imply a similar exposure-induced change in the occurrence of neoplasia. Studies that utilize cell proliferation markers as endpoints are extremely interesting and suggestive, but it remains to be resolved whether inferences about cancer (or neoplasia in general) from these studies are valid. In a prospective study like the Polyp Prevention Trial, it is possible to relate diet to the intermediate endpoint, the intermediate endpoint to adenoma formation, and finally to determine the extent to which any diet-adenoma relation is mediated by changes in the intermediate endpoint.

A positive diet-adenoma finding from this trial, taken in conjunction with the emerging findings on diet and large bowel cancer from existing and planned large cohort studies, will bring us very close to proving a causal link between diet and large bowel cancer, thereby providing a scientific foundation for a practical biologically sound strategy for preventing this disease.

This is a multi-center, randomized, controlled trial involving 2,000 men and women. The projected sample size of 2,000 (1,000 in each of the intervention and control groups) will permit the detection with 90% power of a reduction of 24% in the polyp recurrence rate. Potential participants who have an adenoma removed within three months, meet the eligibility criteria, successfully fill out a series of dietary assessment instruments (a form of "run-in") and complete the informed consent will be randomized into the study. Randomization at each Clinical Center is expected to take up to 2 years. Participants will undergo colonoscopy again at one and four years into the study. Although the recurrence of one or more adenomas is the primary endpoint of the study, it will also be possible to relate the dietary intervention to number, size, and histotype of polyps.

One of the critical (and expensive) components of this trial is intensive nutrition counseling of participants in the intervention group. The general nutrition intervention strategy will integrate the teaching of nutrition skills, self-monitoring techniques, behavior modification techniques, and social support systems. The major strategy for the nutrition counseling will be a step-by-step approach to dietary change based on the needs and abilities of the individual participant. Group counseling will be implemented in the second year of followup. The control group subjects will be provided, if needed, with counseling and education materials on basic nutrition principles for maintaining nutritionally adequate diets, with no emphasis being placed on modification of fat, fiber, or vegetable and fruit intake.

For dietary assessment, food frequency questionnaires, and 4-day diet records will be administered prior to randomization and annually after randomization. In addition, unannounced 24-hour dietary recalls will be administered by telephone to a 10% sample of participants on a yearly basis. Blood specimens will be also be collected for analysis of serum lipids, carotenoids, and other parameters; blood will also be stored for possible DNA analysis.

In order to perform the intermediate endpoint studies, rectal (and possibly other) biopsy specimens will be obtained at baseline and at or shortly before each of the colonoscopic procedures. Both bromodeoxyuridine and proliferating cell nuclear antigen assays will be performed on the biopsy specimens.

Awards for the Data and Nutrition Coordinating Center (Westat, Inc.) and the Clinical Centers were made in September 1990. Randomization started in June 1991 is expected to be completed by the end of 1993.

This study is being conducted collaboratively with scientists from the Biometry Branch, the Diet and Cancer Branch, and the Applied Research Branch of the Division of Cancer Prevention and Control. The Clinical Centers are the University of Pittsburgh in Pittsburgh, PA; the Kaiser Foundation Research Institute in Oakland, CA; the Memorial Sloan Kettering Cancer Center in New York, NY; the State University of New York at Buffalo, NY; the Walter Reed Army Medical Center in Washington, DC; the University of Utah in Salt Lake City, UT; and the Edward Hines Jr. Veterans Administration Hospital in Chicago, IL.

Early Detection Studies:

Biologic Specimen Bank for Study of Early Markers of Lung Cancer Among Tin Miners in Yunnan, China (Z01 CN 00176-02 CPSB)

The primary objective of this study is to establish a biologic specimen bank and data bank that can be used for the validation and refinement of potential early markers of lung cancer. A secondary objective includes the establishment of a cohort for the study of environmental (including dietary) and genetic risk factors for lung cancer.

Lung cancer is the leading cause of death from malignant neoplasms in the United States and many countries around the world, with the incidence of and mortality from this disease still on the increase in most areas. Reduction in the mortality from this lethal malignancy will require reduction in the prevalence of risk factors and/or improved diagnosis and therapy. Although reductions in the prevalence of cigarette smoking in this country promise a future decline in lung cancer rates, smoking is increasing in other countries, particularly underdeveloped countries. Further, persons at high risk of developing cancer due to exposures to other lung carcinogens remain so even when active exposure has ceased. While relative survival rates for localized disease are dramatically better than for nonlocalized disease, most patients are not diagnosed early enough for present therapies to be effective. Over two-thirds of patients with non-small cell lung cancer have clinically detectable regional or distant metastatic disease at presentation. For small cell lung cancer nearly 90% present with nonlocalized disease. Attempts to reduce mortality through early detection by screening with conventional modalities (i.e., chest x-ray and/or sputum cytology) in controlled trials have not demonstrated clear-cut benefit.

Advances in our understanding of the biology of lung cancer in recent years indicate that research to identify early markers of lung cancer may hold great promise for the reduction of lung cancer mortality. Numerous potential candidates for the early detection of lung cancer in sputum exist. Particularly promising among these existing potential early markers are the monoclonal antibodies to small cell (SCC) and non-small cell lung cancer (NSCC). Using prospectively collected sputa from 62 participants in a randomized screening trial, 20 of 22 men who eventually developed lung cancer (91%) had positive immunostaining for either SCC or NSCC an average of 2 years prior to clinical diagnosis, while only 5 of 40 noncases (12%) stained positively.

The Yunnan Tin Corporation (YTC), located in Yunnan Province in southern China, is a large, nonferrous-metals industry, formed in 1883 and nationalized after the establishment of the People's Republic in 1949. It is involved principally in the production of tin from the mines around the city of Gejiu in an area where tin mining dates back 2,000 years.

The tin miners at the YTC have an extremely high rate of lung cancer. Among high risk miners, defined as 40+ years old with 10+ years of underground mining and/or smelting experience, lung cancer rates exceed 1% per year. These extraordinary lung cancer rates result from combined exposure to radon, arsenic, and tobacco smoking in the form of cigarettes and/or bamboo water pipe.

The extremely high incidence of lung cancer among Yunnan tin miners was first recognized in the late 1960's and resulted in the establishment of an annual mass screening program among high risk miners. Since 1973 an average of approximately 7,000 active and retired miners have been screened for lung cancer annually by physical examination, chest x-ray, and cytologic examination of the sputum.

The large population of tin miners at extraordinary risk for lung cancer in combination with the ongoing lung cancer screening program among these high risk YTC tin miners represents a unique opportunity to collect and test biologic specimens, particularly sputum but also blood and urine, for the validation and refinement of early markers of lung cancer. This is also a unique opportunity to prospectively examine a number of potential risk factors such as radon, arsenic, tobacco, diet, and biochemical markers of nutritional status in relation to lung cancer incidence.

Approximately 7,000 tin miners at high risk (a history of 10 or more years of underground mining and/or smelting experience) for lung cancer will have sputum specimens collected and stored annually for five years. Other biologic specimens (blood, urine, and toenails) will be collected and stored during the second year of the study. Followup will be conducted annually during the study to identify all newly diagnosed cancers. Prediagnostic sputum and other biologic samples will be analyzed for potential early markers utilizing a nested case-control approach.

Data collected on all study participants annually will include information collected on the current YTC mass screening form (demographics, medical history, symptoms, smoking history, occupation history, sputum cytology result, chest x-ray result, and physical exam result). This information will be supplemented with a dietary assessment instrument.

Each year all participants in the sputum collection cohort will be followed to determine if any have developed lung cancer. Endpoint ascertainment will rely on existing methods to identify cases. This includes the annual screening examinations and the YTC cancer registry, which was established in 1954 and receives information on all cancers through the YTC Workers Hospital. Because lung cancer is a designated occupational disease at the YTC, case ascertainment is excellent. Diagnostic materials from all cases will be retained for review. At an annual rate of 1%, it is estimated that approximately 70 newly diagnosed cases of lung cancer will be identified annually from among this cohort.

In the first year of the study, 6,378 high-risk miners were identified and screened for this cohort and a total of 88 lung cancers were found. A variety of activities are in the planning or initiation phase, including: evaluation of sputa from cases and noncases from the first year for monoclonal antibodies to tumor-associated antigens from non-small and small cell carcinoma, oncogene studies using archival tissue from lung cancer cases exposed to different occupational carcinogens, assessment of lead^{210} as a measure of cumulative radon exposure, and a dietary history validation study.

This study is being conducted collaboratively with scientists from the Labor Protection Institute of the Yunnan Tin Corporation in Gejiu, China; the Johns Hopkins School of Hygiene and Public Health in Baltimore, MD; the Cancer Institute of the Chinese Academy of Medical Sciences in Beijing, China; and the Biostatistics Branch of the Division of Cancer Etiology.

PLANS

Etiologic Studies:

Many studies of diet and cancer demonstrate inconsistent results. Reasons for these inconsistencies include both difficulties in assessing exposure due to the complexity and variation of our diets, as well as the relatively small increases in risk being estimated. Although dietary assessment methods will continue to be refined, one approach to dealing with the problem of measurement error in studies of small relative risks is to prospectively study very large groups. We are assembling just such a large cohort for studying diet-cancer relations, one that permits the examination of the extremes of dietary intake.

While it is clear that certain malignancies are hormone dependent, the role of hormones in the development of cancer has not been conclusively demonstrated. The development of assays that focus on bioavailable (as opposed to total) hormone levels has opened up new opportunities for evaluating the hormone-cancer relation. We have initiated a study to examine the relation of hormones to breast cancer using prospectively collected serum and are planning similar examinations relating hormones to prostate and other cancers using blood samples from extant cohorts.

The recently completed carotenoid food composition database is being used to estimate the amount of total and specific carotenoids in previously conducted epidemiologic studies. Estimates of specific carotenoid-cancer relations generated using the new database are being compared with estimates from earlier analyses. The results of this activity could lead to the identification of a specific or total carotenoid intake that is strongly related to cancer risk. Based on these efforts, we expect to develop a core set of questions that reflect carotenoid intake across various demographic subgroups in the United States.

A large number of studies have shown that increased body size is associated with an increased risk for cancer, especially cancers of the breast and colon. The direct relation of height to cancer suggests a role for remote nutrition, particularly total caloric intake during growth, in carcinogenesis. Physical inactivity has also been linked to breast and colon cancer. We plan to further explore the hypotheses that body size and physical activity are related to cancer in other study groups and look at potential mechanisms of action. We also plan to examine in detail the relation of diet to body size and breast cancer risk factors among children. Methodologic questions about the assessment of dietary intake in youth via questionnaires administered to adults will also be addressed.

Obesity, weight gain, and the central location of body fat (as opposed to peripheral) have also been associated with increased risk for breast cancer. Further work is planned to evaluate these observations in other groups and to examine potential mechanisms of action.

The alcohol-breast cancer relation is still unresolved. Results from our own studies have been inconsistent, but the majority of epidemiologic evidence still supports the hypothesis. Further, more refined epidemiologic investigations of the question are warranted. The questions of timing of exposure and type of alcohol used need additional study, and plausible mechanisms of action need to be further explored.

One component of the recently-initiated study (Z01 CN 00176-02 CPSB) among tin miners in China is a prospective examination of lung cancer risk factors—including diet, radon, arsenic, and tobacco exposures—as well as genetic factors and micronutrients. Skeletal burden of $lead^{210}$ directly assesses personal exposure to radon, and a unique feature of our examination of risk from radon exposure will be the measurement of $lead^{210}$ in cases and controls. A dietary history validation study among tin miners is in progress.

The CPSB is making plans to participate in a national multi-center cooperative study of the epidemiology of colorectal adenomatous polyps. This study is unique in interviewing and collecting biologic specimens from asymptomatic individuals before they undergo complete colonoscopy. The sample size of 3,000 will yield a substantial number of large adenomas as well as a greater number of smaller lesions. The CPSB will participate in the biomarkers component of this study. Rectal biopsies and bloods (and possibly stool and urine specimens) will be obtained from study participants in addition to the polyp material. Potential biomarkers include rectal epithelial cell proliferation indices, enzyme polymorphisms (assayed in white cell DNA), serum micronutrients, and chromosome alterations/aneuploidy in polyp material. A dietary instrument will be included in the baseline interview.

Family history and familial aggregation have consistently been demonstrated to be risk factors for esophageal cancer, and segregation analyses are most compatible with an autosomal recessive gene. Based on these findings, plans are being made to collect blood for esophageal cancer linkage studies from informative family pedigrees collected in a high risk area in Shanxi Province (see Z01 CN 00150-05 CPSB) to identify the esophageal cancer gene.

Clinical Nutrition Studies:

In addition to assays of bioavailable hormone fractions, other new assays have been developed for metabolic products of estradiol, such as 16-hydroxyestrone. Measurement of these metabolites among subjects participating in controlled dietary studies should allow us to draw conclusions about dietary-induced changes in metabolic pathways that may affect carcinogenesis. Such evaluations can be done in new as well as previously conducted studies.

The effect of diet on hormone levels has been a major theme in our studies of fat reduction among adults, but an area of equal concern is what effect diet has on hormone levels in children. The ancillary study to the DISC study (Z01 CN 00153-04 CPSB) is the first intervention among children in which we have participated. It should offer a number of useful scientific insights on this subject as well as provide us with information on the logistics of handling such studies in children.

It has been reported that women who smoke, drink alcohol, or take oral contraceptives have significantly lower plasma carotenoid levels. It is unknown whether these lower carotenoid levels are due to differences in dietary intake or metabolism. Lower plasma carotenoid levels have, in turn, been related to higher risk of breast cancer. New controlled feeding studies will focus on the effects of hormone use and other lifestyle factors on plasma carotenoids in women.

A number of different elements are present in toenails, and this presents the possibility that analysis of toenails can be used as an objective measure of intake of these elements. Using duplicate plate foods collections and toenails from South Dakota and Wyoming residents collected as part of another study, we plan to perform multi-element analysis of both foods and toenails to determine the relation of intake to toenail levels. A similar evaluation is planned using toenails from Chinese intervention trial subjects before and after several years of regular supplementation with multiple vitamins and minerals.

Prevention Studies:

The ATBC Trial (Z01 CN 00100-11 CPSB) has completed active intervention as scheduled in March 1993. While the primary endpoint of the trial is lung cancer incidence, other cancer and noncancer endpoints will be examined in relation to the intervention. This study is also being viewed as a large, prospective study of diet and cancer in which baseline and at least one interval serum sample have been collected. Other cancer sites of particular interest in this cohort are prostate and stomach. Active planning for followup of this cohort after the intervention phase has been completed is underway.

The Isotretinoin-BCC Study (Z01 CN 00103-11 CPSB) concluded active intervention in June 1990 and the main study results have been published. Future analyses will be directed at the elucidation of risk factors (including diet) for basal and squamous cell skin cancers. In followup of our observed increase in skeletal toxicity in the isotretinoin group, we plan to examine the relation of serum levels of free retinol, retinol binding protein, and prealbumin in subjects with ankylosing vertebral hyperostosis and a comparison group from the trial. Elevated levels may provide a mechanistic explanation for the finding. We also will explore the relation between chronic sun protection behaviors and serum levels of 25-hydroxy vitamin D in a subset of patients.

The Linxian nutrition intervention studies (Z01 CN 00112-10 CPSB) concluded active intervention in 1991, publication of main results is proceeding, numerous evaluations of the relation of the interventions to intermediate endpoints are in progress, and a 5-year post-intervention followup is underway. Based on the protective effects for several of the combination nutrient factors observed in the main trials, plans are being developed now to conduct a new intervention in another high-risk population that will allow us to determine which of the individual nutrients found to be efficacious in the combinations were responsible for the benefits observed.

The Polyp Prevention Trial (Z01 CN 00151-05 CPSB) is nearing completion of randomization. The trial is planned to run through 1997.

There have been very few clinical trials conducted that determine the effect of chemopreventive agents on biological markers or surrogate endpoints in high-risk populations. One such trial currently being planned will involve determining the success of promising new chemopreventive agents in reducing bronchial metaplasia among a small group of tin miners in China, an occupational group with exposure to tobacco, radon, and arsenic, who have lung cancer rates among the highest in the world. Two particularly promising groups of compounds in various stages of testing being considered for use here are the retinoids (synthetic analogues of vitamin A) and the dithiolthiones (purified substances found in a number of the cruciferous vegetables).

Nutritional assessment of the tin miners in Yunnan, China suggests that intake of several micronutrients, most notably selenium and beta-carotene, is low. We are considering the possibility of conducting a nutritional intervention study to prevent lung cancer among several thousand of these high-risk miners.

Early Detection Studies:

Initial evaluations indicate that there is substantial value for balloon cytology in predicting subsequent esophageal cancer among subjects living in high-risk areas for esophageal cancer in Henan Province, China. Plans are underway to further evaluate the usefulness of balloon cytology in identifying precancerous and early cancerous lesions of the esophagus. In addition, we are planning to evaluate new non-invasive methods of treating early esophageal lesions through the endoscope. If these strategies prove to be promising, we will consider conducting a screening trial using these techniques.

CANCER PREVENTION STUDIES BRANCH STAFF

Branch Chief	Philip R. Taylor, M.D., Sc.D.
Deputy Branch Chief and Pharmaceutical Research Coordinator	Joseph A. Tangrea, R.Ph., M.P.H.
Medical Officers	Demetrius Albanes, M.D. Arthur Schatzkin, M.D., Dr.P.H.
Nutritional Epidemiologist	Michele R. Forman, Ph.D.
Nutritionist	Elaine Lanza, Ph.D.
Senior Staff Fellows	Sanford M. Dawsey, M.D. Joanne Dorgan, Ph.D.
Staff Fellow	Lee Chen Yong, Ph.D., R.D.
Research Study Coordinators	William S. Campbell, R.N. Laura Kruse, R.D., M.P.H.
Nurse Specialist	Michelle M. Maher, R.N.
Visiting Associate	You-Lin Qiao, M.D., M.P.H
IPA	Klaus Lewin, M.D.
Secretary	Dawn Smith
Technical Assistant	Nancy Cruz
Stay-In-School	Elena Carter

CANCER PREVENTION STUDIES BRANCH BIBLIOGRAPHY

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LABORATORY OF NUTRITIONAL AND MOLECULAR REGULATION

OBJECTIVES

The overall objectives of the Laboratory of Nutritional and Molecular Regulation (LNMR) can be summarized from its functional statement:

- Plans, develops and conducts intramural basic research on cellular and molecular regulation relevant to nutrition and cancer.
- Performs research studies in biochemistry, cell biology and molecular biology relevant to nutrition and cancer emphasizing the mechanisms by which nutrients, directly or indirectly, augment or inhibit tumorigenesis.
- Formulates and tests heuristic models on nutrition and cancer by studying the absorption and conversion of dietary substances in metabolic studies in animals.
- Investigates the applicability of these models to cancer prevention in humans by measuring plasma or cellular levels of metabolic intermediates, nutrient-derived effector molecules, circulating hormones and/or growth factors in healthy humans undergoing dietary manipulations.
- Contributes scientific back-up to the Institute's programs in research on diet, nutrition and cancer, and serves as a focal point for new information pertaining to nutrition research.

OVERVIEW

Significant progress has been made in the projects initiated by the multidisciplinary staff of the LNMR. We have defined new paradigms for understanding the relationship of dietary factors and nutrients to carcinogenesis. This diet-dependent biology emphasizes the interaction between nutritional and dietary factors and mechanisms of carcinogenesis using cellular and molecular approaches.

Although the elucidation of mechanisms has been emphasized, our efforts focus on scientific leads from clinical epidemiology, intervention studies and studies in animals showing associations between diet and cancer prevention. Our goal is to establish a molecular basis for the effects of diet and nutrition in cancer prevention.

ACCOMPLISHMENTS AND PLANS

A New Mechanism for Carcinogen Resistance: Regulation by Diet and Nutrients
(Z01 CN 00155-03 LNMR)

The concept of cellular resistance to carcinogens based on cellular efflux mechanisms has not been previously invoked. We proposed that the efflux pump, mediated by a plasma membrane glycoprotein (P-gp) and coded for by *mdr* 1, not only confers resistance to pleotropic

cytotoxic drugs but also to chemical carcinogens. Using a series of multi-drug resistant (MDR) human breast cancer cells (MCF-7) with different stages of adriamycin resistance which correlated with expression of P-gp, we showed that resistance to benzo(a)pyrene is correlated to its cellular efflux mediated by P-gp. Findings supporting this hypothesis included: 1) Increased IC_{50} for benzo(a)pyrene corresponding to increased P-gp levels; 2) Increased rates of benzo(a)pyrene efflux corresponding to increased P-gp levels; 3) inhibition of benzo(a)pyrene efflux with verapamil, a known inhibitor of the P-gp mediated efflux pump for cytotoxic drugs and 4) benzo(a)pyrene inhibition of azidopine binding to P-gp.

Having established that cellular resistance to benzo(a)pyrene can be mediated by P-gp, we then extended this effect to other carcinogens, e.g., 7,12-dimethylbenzanthracene (DMBA). We showed that this well known experimental carcinogen is accumulated to a lower level in cells expressing P-gp and the efflux of DMBA is at a higher rate in these cells. These findings support our hypothesis that the efflux of certain carcinogens can be mediated by P-gp and suggest that P-gp may play a role in protecting normal cells from chemical carcinogens.

A major goal of this project is to identify dietary and nutritional mechanisms for modulating the expression and function of the P-gp mediated efflux of carcinogens. Although P-gp expression in cultured cells can be enhanced by retinoic acid and sodium butyrate, their role in modulating P-gp expression or function remains unclear. We screened a variety of naturally-occurring compounds and found that flavonoids, a widely distributed constituent of fruits and vegetables, decreased the accumulation of adriamycin. Using MDR MCF-7 cells, we then showed that these flavonols, e.g., galangin, quercetin and kaempferol, markedly increased the efflux of DMBA and decreased its accumulation through a P-gp mediated mechanism. We propose that P-gp mediated carcinogen efflux may be regulated by dietary factors in normal tissues and is an important mechanism linking dietary fruits and vegetables to decreased cancer risk. The molecular mechanism for this effect and its implications on carcinogenesis are being investigated.

Protection Mechanisms Against Endogenous Carcinogens and Nutritional Regulation in Placenta-Related Cells (Z01 CN 00156-03 LNMR)

The plasma membrane glycoprotein 170 (P-gp) responsible for multidrug resistance (MDR) also may function as a efflux pump for chemical carcinogens and can be regulated by diet and nutrients. P-gp is found predominantly in the cells lining the luminal space of a variety of normal tissues, including the placenta and the endometrium of the gravid uterus. The functional role of P-gp in normal tissues has not been determined. However, our recent findings indicate that P-gp mRNA is developmentally regulated in human placental tissues. Currently, a developmental study in rats of P-gp expression in normal tissues during gestation is in progress. Rat placentas, ovaries, uteri, adrenals and kidneys at 0, 6, 9, 12, 15 and 18 days of gestation are being processed for P-gp expression at the mRNA and protein levels. In addition, a SV40-temperature sensitive *A* (*tsA*) rat placental cell line is used to examine the effects of nutrients and diet factors in P-gp regulation.

A doxorubicin-resistant rat placental cell line has been established as a model for examining P-gp function in the defense against carcinogens. We will study the regulation of P-gp by nutrients and carcinogens in wild-type and drug-resistant placental cells at the protein and mRNA levels. In addition, we are studying the regulation of P-gp in a human endometrial adenocarcinoma cell line and a human cervical carcinoma cell line. Dietary effectors such as retinoic acid, known to enhance P-gp expression, and the flavonoid, kaempferol, are being investigated in the human endometrial and cervical cell lines. Special attention is paid to the two forms of MDR genes (*mdr* 1 and *mdr* 3) and their differential regulation by dietary factors and carcinogens in order to understand the physiologic regulation of P-gp.

The Effect of Proteins, Peptides and Amino Acids on Carcinogenesis and Tumor Progression (Z01 CN 00157-03 LNMR)

Renewed interest in protein intake and cancer incidence has focused on qualitative differences in proteins from various sources as well as on the contribution of proteins to total caloric intake. We proposed a novel mechanism mediating the effect of proteins on mitogenesis and carcinogenesis. Our hypothesis is based on the known modulatory effects of proline and its oxidized intermediate, pyrroline 5-carboxylate, on metabolic events in post-receptor signaling. Additionally, imidodipeptides, dipeptides with proline or hydroxyproline at the carboxyl terminus, are delivered to tissues and hydrolyzed by a specific enzyme, prolidase. We showed that these imidodipeptides can serve as an important source of proline for cells. A cultured cell line auxotrophic for proline attained maximal growth rates on GLY-PRO in the absence of added free proline. This finding showed that dietary sources of protein supplying different levels of imidodipeptides could differentially deliver proline to tissues. Since imidodipeptides are also the product of matrix collagen degradation, prolidase serves as an interface between protein nutrition and matrix breakdown. Our studies suggested that the level of cellular prolidase is regulated by extracellular collagen acting through integrin receptors. Thus, the hydrolysis of imidodipeptides, the final degradative products of matrix collagen, is responsive to cellular interaction with extracellular matrix. We are studying the regulation of this enzyme on the molecular level.

These regulatory mechanisms involving proline and pyrroline 5-carboxylate may be especially relevant to prostate tissues. Since prostate epithelial cells are known to exhibit special metabolic features, e.g., the secretion of large amounts of citrate derived presumably from aspartate, the metabolism of amino acids and the regulation of the redox and energy states are critically important. Additionally, the conversion of testosterone to dihydrotestosterone, a redox-dependent enzymatic step, is considered critical for progression of prostatic cancer. The linkage of proline and pyrroline 5-carboxylate to these metabolic and redox features may be specially important for prostatic cancer. We have shown that there is a decreased P5C stimulated flux in the androgen sensitive prostate cancer cells. We will investigate the importance of the proline cycle and pyrroline 5-carboxylate-mediated events in both rat prostate tissues and in normal and malignant cultured human prostate cells.

Finally, the gene for human pyrroline 5-carboxylate reductase has been cloned. This enzyme is critical in the regulatory effects of pyrroline 5-carboxylate. We are raising polyclonal antibodies to peptides based on the deduced sequence. These antibodies will allow studies on enzyme localization and on mechanisms linking pyrroline 5-carboxylate to its modulation of post-receptor signaling. We are also using the cDNA to examine regulatory mechanisms for expression of P5C reductase. Preliminary studies have shown qualitative and quantitative differences in P5C reductase mRNA between the androgen-sensitive and insensitive prostate cancer cell lines.

An *in vitro* Model to Assess Biochemical and Molecular Biological Effects of Dietary Fiber on Cancer Prevention (Z01 CN 00161-03 LNMR)

Epidemiologic studies suggest a lowered risk of hormone-dependent cancers among vegetarians. Vegetable and fruits contain lignans and isoflavones which can be converted to biologically active hormone-like substances by intestinal flora. The interaction of these compounds with endogenous hormones has not been evaluated and may be an important mechanism in cancer prevention. We have established an assay system that identifies estrogenic factors in the diet. We used MCF-7 human breast cancer cells since it is known that the transcription of the pS2 gene is directly controlled by the action of estradiol in this estrogen receptor positive cell line. The expression of pS2 RNA was monitored by Northern blot analysis using a non-radioactive DIG-labeled probe. The effect of various phytoestrogens including enterolactone, enterodiol, equol, nordihydroguiaretic acid (NDGA), genistein, kaempferol, daidzein and quercetin on pS2

expression in MCF-7 cells were studied. From our results, it appeared that equol, genistein, daidzein and kaempferol are able to elicit a strong pS2 response; enterolactone evokes a milder response while quercetin and enterodiol are inactive. The effect of these different compounds on cell growth corroborated their estrogenic effects on pS2 expression.

The ability of enterolactone, quercetin, genistein, NDGA and equol to compete with estradiol for binding to the estrogen receptor was also evaluated. Genistein, equol, NDGA competed with estradiol for binding to its receptor while enterolactone and quercetin did not.

We have devised a sensitive assay to assay extracts of fruits and vegetables as well as to test various diet-derived components for estrogenic activity. Furthermore, we are planning to study the effect of various compounds on other cell lines derived from tissue other than breast. In addition, we will emphasize the mechanism of action of lignans and flavonoids at the molecular level.

Mechanisms for Deranged Androgen Responsiveness in Prostate Cancer Cells
(Z01 CN 00163-02 LNMR)

Androgens, testosterone and its metabolites, play a large role in the normal growth and function of the prostate. However, changes in androgen metabolism or responsiveness to androgens have been implicated in the formation of benign prostatic hypertrophy and prostate cancer. The causes of these changes are not well understood. Studies were undertaken to determine what if any differences in androgen metabolism occur between a) androgen-dependent and b) androgen-independent prostate cancer cells.

Whole cell studies showed that in androgen-dependent cells, added testosterone is primarily glucuronidated. The cellular level of total testosterone, i.e., testosterone plus its glucuronide, remains constant during the entire incubation period. The kinetics, regulation, and physiologic effect of glucuronidation is being investigated. Androgen-independent cells, on the other hand, metabolize testosterone predominantly to androsterone. Unlike the dependent cell line very little androgen remains within the cell at the end of the incubation period. Assays in a cell-free system show markedly lower UDP-glucaronyl transferase activity in these cells as compared to the androgen-dependent cells.

Our goal is to elucidate the mechanisms responsible for these differences in androgen metabolism and to determine how dietary factors modulate these mechanisms.

Mechanisms of Caloric Restriction in Cancer Prevention (Z01 CN 00178-01 LNMR)

Elucidation of the cellular/molecular mechanism(s) of caloric restriction (CR), which inhibits the development of a variety of spontaneous and experimentally-induced tumors in rodents, may provide important clues for human cancer prevention. We are using a p53-knockout transgenic mice as an *in vivo* model to explore the mechanisms underlying the anti-tumor effects of CR.

The p53 gene is the most commonly identified mutated gene in human tumors. Recent evidence suggests that the wild-type p53 gene product protects cells against mutations by mediating an arrest of the cell-cycle in response to DNA damage, facilitating repair of damaged DNA and preventing fixation of mutagenic lesions that can lead to neoplasia. Mice with the p53 gene knocked-out by homologous recombination develop normally but have increased susceptibility to spontaneous tumor development, with approximately 100% tumor incidence by 6 months of age in untreated homozygous p53-deficient mice. We have shown that CR markedly decreases the incidence and increases the latency of spontaneous tumor development in these mice.

Cellular and molecular studies on tissues collected serially from wild-type, homozygous and heterozygous p53-deficient mice fed *ad libitum* or CR-treated, are currently in progress. These include analyses of cell-cycle, immune competence and DNA repair. Data from early timepoints using lymphocytes isolated from treated animals indicate that p53-deficiency accelerates cell-cycle traverse and that caloric restriction in p53-deficient animals significantly decreases cell cycle traverse. We have also begun analyzing tissues for differences in p53, *ras*, *fos/jun* and *mdr* expression using Northern blot analysis.

We are also conducting a 2-stage skin tumorigenesis experiment with wild-type and heterozygous p53-deficient mice to determine the stage in the carcinogenesis pathway in which CR is exerting its effects and to further explore the mechanisms underlying the anti-tumor effects. The effect of caloric restriction in the presence and absence of the p53 gene on the development of papillomas and the progression of papillomas to carcinomas will be evaluated.

Finally, we have established *in vitro* embryonic fibroblast cell lines isolated from wild-type, homozygous and heterozygous p53-knockout mice to facilitate the evaluation of potential intermediates of the tumor-inhibitory effects of CR.

Effects of Vitamin A Nutriture and Synthetic Retinoids on Retinol Metabolism
(Z01 CN 00162-03 LNMR)

The cancer chemopreventive and chemotherapeutic role of retinoids has been demonstrated in a variety of studies. However, the clinical usefulness of retinoid therapies is not likely to be fully realized until basic aspects of their metabolism are better understood. First, we investigated at the molecular, tissue and whole body level the mechanisms involved in the normal physiological metabolism of vitamin A. Secondly, we examined the effects of chemopreventive retinoids on normal vitamin A metabolism. Several long-term studies of retinol kinetics were performed in animals fed N-[4-hydroxyphenyl] retinamide (4-HPR) or all-*trans*-retinoic acid. Following IV injection of a physiologically radiolabeled dose of retinol, retinol tracer and tracee kinetics were monitored in plasma and tissue for up to 41 days. Kinetic parameters were determined using the SAAM/CONSAM computer modeling programs to carry out graphical analysis of tracer concentration curves. Analysis of data from the 4-HPR study demonstrated major alterations of "native" vitamin A metabolism. Mean plasma retinol levels were reduced in the 4-HPR group to one-third of controls. The fraction of the plasma retinol catabolized per day was nearly twice as high in the 4-HPR treated group. The amount of time that retinol molecules spent in the plasma before being lost from the system was cut nearly in half in the 4-HPR treated group and the amount of vitamin A retinol ultimately utilized in these animals was 33% less than that used by the control group. Studies investigating the mechanisms by which 4-HPR alters retinol kinetics are present underway in our laboratory. The results thus far would suggest that long-term administration of 4-HPR markedly perturbs normal retinol metabolism in rats. Whether 4-HPR similarly alters human retinol metabolism with untoward clinical consequences deserves careful evaluation. We are presently developing the appropriate methodology using stable isotope forms of vitamin A to carry out turnover studies in humans.

With the possibility of future studies in humans, we have begun to develop appropriate methodologies. To this end we have used the polymerase chain reaction to construct an *E. coli* expression plasmid that allowed for the expression of human plasma retinol-binding protein (RBP). Purification and characterization of the expressed RBP have been completed and preliminary studies suggest this protein is functionally active. Thus, with the addition of a stable isotope form of retinol to the expressed protein, it is possible that one could conduct retinol turnover studies in human subjects.

Dietary Lipids and Signal Transduction in Breast Cells (Z01 CN 00158-03 LNMR)

A potential link between nutrients, dietary factors and human cancers involve fat consumption and the increased risk of breast and colon cancer. A cell culture model using tumorigenic and nontumorigenic human breast cells has been established to study the role of fat, cellular membrane lipids, and the derivation of lipid mediators in signal transduction mechanisms that regulate mitogenesis of breast cells.

One line of investigation has determined that MCF-7 human breast cancer cells lack the ability to synthesize ether-linked phospholipids. The evidence indicates the deficiency results from a lesion in the alkyl dihydroxy acetone phosphate synthase enzyme system, and the defect appears to be a characteristic of the MCF-7 cell line. In light of previous work suggesting that ether lipids contribute to neoplasia, this cell model represents an experimental system for defining the role of ether lipids in signal transduction processes and in the development of neoplastic behavior.

A second series of experiments has determined that multidrug resistant MCF-7 cells possess increased tumor promoter-stimulated phospholipase D activity (about 4x over drug sensitive cells). To characterize this phospholipase activity, we are using gas chromatography/mass spectrometry to provide detailed qualitative and quantitative information regarding the molecular species of the phospholipase D-generated lipid mediators. Related studies are also investigating the effects of growth factors and mitogenic hormones on the phospholipase signaling mechanisms of the breast cells. The breast cell model is amenable to modifying the cellular membrane lipids with dietary fats. Thus, the cell model permits experiments that will define the role of dietary fats as relates to the phospholipase D signaling pathway and will more specifically define the role of lipid mediators as modulators of cell growth.

Regulation of Tumor Suppressor Protein p53 (Z01 CN 00159-03 LNMR)

We have previously shown that mutation of phosphorylation sites Ser 315 and Ser 392 to alanine did not alter the ability of p53 to inhibit the growth of tumor cell line SW480. It was proposed that p53 may serve as a transcriptional enhancer. To obtain more insight into the molecular events, we utilized a transfection assay which monitors the expression of a reporter gene, chloramphenicol acetyl transferase (CAT) to study the effects of mutated phosphorylation sites on p53 function. We found that removal of phosphorylation sites did not affect the ability of p53 to stimulate production of the CAT enzyme. Thus, it appears that phosphorylation of Ser 315 or Ser 392 does not directly modulate the functional activity of p53. Others have reported that accumulation of p53 protein accompanies the cellular response to DNA damage. It is possible that phosphorylation regulated by external stimuli may affect p53 function indirectly by stabilizing the protein and allowing for its accumulation. We are in the process of exploring the possible effects of phosphorylation on p53 stability using *in vitro* and *in vivo* methods. In addition, we are also in the process of examining the effects of dietary compounds which may modulate p53 accumulation.

Nutritional Regulation of *Ras* Proto-Oncogene Activity (Z01 CN 00160-03 LNMR)

Ras-mediated escape from normal regulation appears to be a frequent event in the multistep genesis of cancer. A number of *in vitro* studies have demonstrated interactions between *ras* and other proto-oncogene products, especially *myc* and the tumor suppressor p53. A useful model for the study of these interactions is the commercially available p53 "knockout" mouse, a transgenic mouse in which null p53 germ line mutations prevent the expression of either one or both alleles for p53. Such p53-deficient animals develop normally but are prone to early tumorigenesis; indeed, 75% of homozygotes develop spontaneous neoplasms by 6 months of age.

These mice can be used to study the effects of p53 gene dosage and various diets on carcinogenesis, or they can be used as a model of accelerated carcinogenesis that does not require exposure to chemicals that initiate and/or promote tumorigenesis. Moreover, fibroblasts cultured from embryos with the various genetic backgrounds afford a powerful *in vitro* system for addressing some of the same issues. We will use cDNA probes for *ras, myc* and p53 to assess the expression of these proto-oncogene mRNAs in various tissues from transgenic mice. Studies in progress with Dr. S. Hursting are investigating the effect of caloric restriction (a potent but poorly understood dietary regimen that dramatically inhibits tumor development in rodents); these studies will determine how this dietary manipulation combines with the gene dosage of p53 to affect proto-oncogene expression. Another area of interest is the dietary effect of the monoterpene, limonene, a major component of orange oil that has been shown to inhibit tumorigenesis in animal models. Part of the action of limonene may be through its inhibition of the farnesylation of *ras* proteins; this post-translational lipid modification is required for location of *ras* to the plasma membrane and hence *ras* activity. Using the sensitive semi-quantitative assay for *ras* we previously developed, we will be able to examine the effects of limonene on *ras* in p53-deficient mice *in vivo* and *in vitro*.

LABORATORY OF NUTRITIONAL AND MOLECULAR REGULATION

STAFF

Branch Chief	James M. Phang, M.D.
Senior Investigator	Grace C. Yeh, Ph.D.
Senior Staff Fellows	Kevin C. Lewis, Ph.D. Susan N. Perkins, Ph.D. Cathie A. Plouzek, Ph.D. Neeraja Sathyamoorthy, Ph.D. Thomas Wang, Ph.D.
Cancer Prevention Fellow	Steve Hursting, Ph.D.
IRTA Fellows	James W. Critchfield, Ph.D. James P. Henry, Ph.D. Xia-Ya Sun, Ph.D.
Visiting Fellow	Joanna Lopaczynska, M.D.
Visiting Scientist	Xianmao Luo, M.D., Ph.D. Jerzy Palka, Ph.D.
Chemist	Sylvia J. Downing
Biological Lab Technician	Charles M. Poore
Secretary	Iva C. DeArmon
Volunteer Worker	Nafisa Farah
Summer Student	Matthew Segretti

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BIOMARKERS AND PREVENTION RESEARCH BRANCH

The Biomarkers and Prevention Research Branch, composed of the Office of the Chief and the Experimental Biochemistry Section, was administratively approved in early 1991. Three additional Sections (see Figure 1, page 2) are in the process of being approved. An off-campus unit, the Branch—including both administrative and laboratory activities—has recently moved to a new home at the Key West Life Science Center in Rockville, Maryland. The BPRB has recruited a number of talented investigators and support staff and is proceeding to address the mandate to rationally apply specific information about the nature of early events in epithelial cancer for the development of new and effective tools to effect the detection and intervention of early cancer.

OBJECTIVES

The objectives of the Branch are summarized in its functional statement:

- "Plans, develops, and conducts intramural research to identify new tools for the early detection of epithelial cancers;
- establishes tissue banks which permit validation of new early detection and intermediate end point markers;
- validates the utility of intermediate end point markers which could be used in carefully designed cancer intervention trials;
- trains clinical and basic science professionals who are interested in rational prevention development;
- facilitates the development of technologies which enable large scale screening and prevention trials;
- coordinates activities with other divisional elements to facilitate clinical trial development and to achieve interaction with relevant groups;
- plans, develops, and conducts intramural research in the biochemical pathways of neuroendocrine and epithelial cancers;
- identifies *in vitro* and *in vivo* tumor model systems which allow systematic evaluation of pathways that are amenable to specific biochemical inhibition;
- evaluates specific tumor inhibitors from *in vivo* and *in vitro* studies which would lead to FDA approval for clinical trials;
- interacts with other NIH elements to conduct multidisciplinary clinical and basic science projects to identify tools to control epithelial cancer while it is still confined to the epithelium."

OVERVIEW

Evaluation of the regulation of neoplastic epithelium

The Biomarkers and Prevention Research Branch is interested in identifying fundamental biochemical and/or molecular pathways which are central to the early stages of the development of a cancer. Elucidation of relevant biomarkers which reflect aspects of such a central pathway would lead to further analysis to validate the utility of the biomarker as a tool for early cancer

detection. Examples of efforts to identify critical regulatory steps are provided. Dr. Birrer's group (proposed Molecular Mechanisms Section) is studying the biology of early activation factors and how these molecular events participate in the early stages of tumor promotion. Dr. Jakowlew, along with recently appointed Experimental Biochemistry Section Chief, Dr. Terry Moody, are defining the role of TGF-β in the regulation of lung cancer growth.

Members of the proposed Intervention Section are collaborating with Dr. Marti Jett of Walter Reed Research Institute to evaluate the specific signal transduction path common to growth factor mediated proliferation. As we have recently presented, the activity of the lipoxygenase pathway seems to be centrally involved in these processes and therefore comprises an attractive target for intervention approaches.

Studies of early activation events in patients with "epithelium at risk"

To obtain relevant clinical materials, a protocol will be developed to systematically study the remaining organ at risk in (or normal tissues removed from) individuals who have undergone curative treatment for lung, colon, breast or ovarian cancer.

Individuals who have experienced a cancer at any of these sites still have an increased risk of developing subsequent primary cancers in the same or a related site. We propose to accrue and serially follow a cohort of these patients to obtain relevant followup material (for example, with sputum specimens, breast aspirates, or endoscopic or bronchoscopic biopsies) so that genetic and biologic marker expression can be catalogued. When possible, their results will be compared to the biologic characteristics and molecular phenotype of the patient's original tumor. Research and development for new early detection tools requires ongoing access to such material. In addition, an understanding of the early genetic events in the development of epithelial cancers may suggest preventative intervention strategies. As part of the monitoring protocol, prompt referral of protocol subjects to appropriate oncologic specialists when new cancers are detected by the laboratory analysis will be a required aspect of the study.

The prospective validation of our previously reported early lung cancer detection approach is being conducted at 11 participating university centers through a CRADA mechanism with Johns Hopkins University, the University of Pennsylvania, and Abbott Labs. The final results of this trial should be available in four years but approximately 400 subjects have already been accrued to this study in the second year since its inception.

Use of photodynamic laser therapy to control early lung cancer

If an early detection tool (sputum immunocytochemical analysis) is shown to be effective in screening for individuals with early stages of lung cancer, individuals with cancer confined only to the epithelium of the lung airways would be identified much more frequently. Currently, no approach is available for treating the disease at this early phase. However, one can administer porphyrin compounds, which are taken up in rapidly dividing tissues, and couple that with exposure to laser light to destroy areas of cancerous cells in lung airways. The laser light excites the porphyrin in the tissue and preferentially kills the malignant cells. This may be beneficial, since this approach could destroy foci of early cancer, particularly those that can arise in areas of chronic injury. Conceptually, this would be similar to removing colon polyps in an individual being routinely screened due to a high risk for colon cancer.

Scientists at the Los Alamos National Laboratories have developed a new class of porphyrin that is copper-based rather than iron-based. In collaboration with this group and other NIH groups, we propose to do a preliminary clinical evaluation of one of these new compounds to determine its utility in photodynamic laser therapy of the airway.

We are developing this compound because of the advantage it affords in that the copper in the porphyrin ring can be replaced with radioactive copper. This radiolabeled compound allows localization of the porphyrin within the airways. Investigators from the Biomarkers Branch and the NIH Nuclear Medicine Department have collaborated before to do this type of analysis: to define the localization of monoclonal antibodies in lung tissue. If successful, this analysis may provide another effective therapeutic approach to early lung cancer. Through the use of Nuclear Medicine techniques, direct airway delivery of this copper porphyrin carrying a very high energy radionuclide could be used to directly destroy the potentially cancerous sites within the lung airway. The accuracy of this approach could be further refined by coupling the copper porphyrin to monoclonal antibodies that would then specifically bind areas of carcinogenic injury.

This challenging research project requires research strength in several diverse areas from several different groups. Fortunately these groups already exist at the NIH and Los Alamos and have a track record of effective collaboration.

Pilot study of peptide growth factor antagonist for lung cancer intervention

Investigators of the BPRB have been central to the identification of gastrin releasing peptide (GRP) as a potential tumor promoter in lung cancer. A specific monoclonal antibody to GRP has been used in a clinical trial to determine if neutralizing the growth factor is of clinical benefit. Due to progress in the field of protein chemistry, a peptide antagonist which we have shown to block growth of small cell lung cancer cells in nude mice is now available for testing in humans. This will provide an opportunity to evaluate the relative benefits of a peptide antagonist versus monoclonal antibodies to determine which is more suitable for application as a rational intervention agent. To facilitate pharmacologic analysis, this study would also involve the use of tracer radionuclide doses to permit precise analysis. Radionuclide imaging work would be done in collaboration with the NIH Nuclear Medicine Department, which participated in the previous clinical trials analysis of radiolabeled GRP monoclonal antibodies.

Pharmacology of established chemopreventional agents

Studies have recently suggested a role for tamoxifen and 13-*cis*-retinoic acid as chemoprevention agents for breast cancer and upper aerodigestive cancers (head and neck, esophagus, and lung cancer), respectively. To date, the critical information regarding the lowest possible effective dose as well as the heterogeneity of drug metabolism in the general population have not been established. In collaboration with pharmacologists from the Food and Drug Administration, investigators from the BPRB will intensively study a small number of subjects to establish the optimal biologic dose to permit interference with estrogen stimulation of breast carcinogenesis. In addition, a practical approach to ensuring adequate dosing will be developed by monitoring drug metabolites in blood or urine. Finally, a similar analysis will be done of 13-*cis*-retinoic acid and its effects on squamous epithelium.

The chemoprevention and evaluation approach to cancer will require long periods of therapy. The need to give enough drug to have the desired biologic effect but not excessive amounts of drugs such that a subject experiences side effects imposes a very challenging requirement of this field. The basis for success arises from a rational dosing schedule validated by direct clinical observation. The initial efforts for such rational pharmacologic dosing would be focused on tamoxifen for breast cancer prevention and 13-*cis*-retinoic acid for upper aerodigestive cancers.

AREAS OF RESEARCH EMPHASIS

The Molecular Mechanisms of Oncogene Action (Z01 CN 00164-02 BPRB)

Recent developments in the application of molecular biology to epithelial cancers have led to the identification of specific genetic lesions resulting in either activation or inactivation of key target genes. These genes, called oncogenes, are involved in various aspects of cell growth regulation and as such play major roles in the early carcinogenic processes of "initiation" and "promotion." It is now critical to understand the precise mechanisms by which these genes function so molecular or pharmacologic agents can ultimately be derived to alter or repress their effects.

The purpose of this project is to elucidate the biochemical and molecular mechanisms by which oncogenes transform mammalian cells. To this end, we have performed structure/function analysis on members of the *myc, jun* and *fos* oncogene families. These studies have revealed various structural aspects of these proteins which are necessary and sufficient for transformation.

Our studies of the *c-jun* oncogene revealed that in addition to the DNA binding and dimerization domains, the N-terminal transactivation domain is required for cellular transformation. In addition, the ability of *c-jun* to transactivate correlates with its ability to transform cells. Thus, *c-jun* appears to transform cells by regulating gene expression. Further, detailed mutation analysis of *c-jun* has demonstrated that phosphorylation of cJun at serines 63/73 results in increased transactivation and ultimately transformation. The phosphorylation of these sites occurs in part through a *ras/raf* dependent pathway which provides an important biochemical link between these oncogenes. More recent studies are aimed at a more detailed analysis on other *c-jun* post-translational modifications and their biochemical and biologic effects and parallel studies with the *c-fos* oncogene examining the relationship between phosphorylation and biologic activity.

Our studies of the *myc* oncogene have focused on comparing the transactivating and transforming activities of the *c-myc* and *L-myc* genes. By exon shuffling, we have demonstrated that *L-myc* transactivates and transforms much less efficiently than *c-myc* and this difference is localized to the second exon. More recent work has focused on the precise structural differences between these genes and their role in apotosis.

The Use of Transcriptional Factors as Targets and Agents for Chemoprevention (Z01 CN 00165-02 BPRB)

Transcriptional factors are critical regulators of gene expression. It is clear that these factors control the expression of many genes and as such mediate the biologic effects of agents such as "tumor promoters."

The purpose of this project is to design mutants of transcription factors specifically aimed at inhibiting their biochemical and, most importantly their biologic functions. The AP-1 complex has been specifically implicated in mediating the biologic effects of the tumor promoters "phorbol esters." A major component of this complex is the *c-jun* oncogene. We have created a panel of dominant-negative mutants of *c-jun* which are able to inhibit the biochemical functions of this oncogene. These mutants include:

1) a transactivation mutant with a deletion of amino acids 2-122,
2) three DNA binding mutants including one with a point mutation at position 265, a deletion at positions 269-272, and one with an insertion of 3 amino acids at position 265,
3) a dimerization dependent mutant missing the Leucine zipper,
4) a transactivation mutant (deletion of amino acids 2-122) with a homodimerization domain only, and
5) a transactivation mutant with a heterodimerization domain only.

We have recently begun to test the ability of these mutants to inhibit biologic functions. A transactivation mutant has been shown to inhibit *Jun* and *Fos* oncogene transformation in addition to the *in vitro* transforming effects of the tumor promoter TPA. Further work with this mutant has demonstrated that it can inhibit a wide range of oncogene transformation, the effects of phorbol ester in *in vivo* model systems of "tumor promotion," and tumorgenicity of some mouse epidermal tumor cell lines.

Future efforts are aimed at further refining the potency and specificity of these mutants by creating smaller mutants with higher affinities for dimerization and DNA binding, and testing them in specific human tumor systems such as breast and lung cancers. In addition, we are designing delivery mechanisms which might make these agents more clinically applicable. Finally, we are expanding these studies to include other transcription factors such as CREB.

Role of Transcription Factors in Breast Epithelial Cells (Z01 CN 00179-01 BPRB)

Over the last year, we have begun to investigate the role of nuclear transcription factors in controlling proliferation and transformation of human breast epithelial cells. We have demonstrated that the Jun and Fos families of transcription factors are expressed in a variety of non-tumorigenic and tumorigenic breast epithelial cell lines, and have shown that Jun and Fos RNA expression, and transcriptional activating activity are stimulated by a variety of growth factors, and also by TPA. In addition, an inhibitor of Jun and Fos transactivating activity which effectively suppresses transcriptional activation in rat fibroblasts also inhibits Jun and Fos activity in human breast epithelial cells. Studies are now ongoing to determine if this inhibitor is capable of inhibiting the proliferation or transformation of these human breast epithelial cells.

Additional ongoing studies include the characterization of other transcription factors in human breast epithelial cells. We are presently studying the members of the CREB family which regulate the cellular response to cyclic AMP, and the C/EBP family which are involved in regulating the cellular response to calcium. A detailed characterization of their expression and activity in human mammary cells will allow us to determine the relative role of each of these transcription factor families in controlling cellular proliferation and transformation. Once the activities of these transcription factors are well characterized, we will modulate their activity using inhibitors specific for each family of transcription factors. By interfering with transcription factor function, we may be able to block signal transduction pathways at a distal point where the signals from multiple growth factors converge. If these specific transcription factor inhibitors affect proliferation or transformation in human breast cells, such inhibitors might be promising chemopreventative agents.

Evaluation of Markers for the Early Detection of Breast Cancer (Z01 CN 00180-01 BPRB)

While mammography provides a method for the early detection of breast cancer, there are still breast cancer patients who do not have mammographically detectable lesions. Furthermore, only 25% of women who develop breast cancer have a recognized risk factor. Evaluation of the ductal epithelium of the breast may reveal markers which could identify women who are at an increased risk for developing breast cancer and thereby would derive greater benefit from surveillance or would be appropriate for intervention studies. Breast ductal epithelium is shed into ductal fluid, and this fluid can be aspirated from the nipple in approximately 50-60% of women. Published studies by Petrakis and others have shown that the ability to yield fluid is associated with an increased risk of breast cancer compared to non-yielders, but proliferative cytology alone is not adequate to predict breast cancer occurrence.

Evaluation of biomarkers on the breast epithelium detectable in breast duct aspirate may provide a method of early detection of cellular changes. During the summer of 1993 a protocol will be submitted for a feasibility trial to determine the acceptability of obtaining breast nipple aspirate fluid, to characterize the range of volumes obtained and to develop methods of

performing multiple assays of the fluid obtained. Specimens will be examined for expression of markers including IGF-1 or TGF-β levels, estrogen and progesterone receptors, retinoic acid receptors, erbB-2 expression, carbohydrate antigen expression and others. Breast duct aspirate and breast needle aspirates would be obtained to characterize marker expression detectable in one or both specimen sources for concordance. An intervention trial with tamoxifen is planned in a very high risk population identified by traditional risk factors to determine whether marker expression changes with tamoxifen administration. Serial specimens would be examined for modulation of marker expression in response to the intervention. Pharmacologic investigations are proposed to examine the lowest dose of these agents which are associated with a biologic effect.

In addition, women with abnormalities on screening mammography would be stratified by whether biopsy of the abnormality was recommended based on standard clinical practice. The characterization of the association between mammographic findings and biomarker expression should be a useful adjunct to the management of these women.

The Molecular Genetics of Gynecologic Cancers (Z01 CN 00181-01 BPRB)

Gynecologic cancers remain a major problem in this country with approximately 25,000 deaths annually attributed to these diseases. The purpose of this project is to characterize the molecular genetics of this group of tumors and ultimately use that information for clinical applications in designing therapeutic and prevention trials

We have characterized a group of ovarian tumors which span the histologic spectrum from benign cystadenomas through tumors of "low malignant potential" (LMP) to ovarian carcinomas for mutations in the *ras* and p53 oncogenes. Results from this study revealed that while benign and LMP tumors possessed activated *ras* genes, ovarian carcinomas did not. In addition, mutations in p53 were frequent in ovarian carcinomas but not in LMP tumors. This suggests that these tumors are discrete biologic entities.

We are also determining the molecular "signature" of uterine sarcomas and leiomyomas, and various endometrial specimens which span the histologic spectrum from benign to malignant. These studies will help to identify the molecular genetic events which are important in the genesis of these tumors. In addition, it will characterize the temporal relationship among these events enabling one to determine if any of these lesions can be used as markers of early disease.

Expression of TGF-β Isoforms in Human Lung Cancer Cells (Z01 CN 00166-02 BPRB)

Objectives:

This project on expression of transforming growth factor (TGP) is directed toward five goals: 1) identifying TGF-β mRNAs in non-small cell lung cancer (NSCLC) cells and small cell lung cancer (SCLC) cells; 2) identifying TGF-β proteins in lung cancer cells; 3) correlating TGF–β mRNA expression with TGF-β protein expression using Northern blot hybridization and immunohistochemical staining techniques; 4) identifying retinoic acid receptor (RAR) and retinoid X receptor (RXR) mRNAs in NSCLC and SCLC cells; and 5) examining the interaction of TGF-β with retinoic acid to investigate whether retinoic acid can be used to increase expression of TGF-β and slow proliferation of lung cancer cells.

Methods Employed:

Standard methods were utilized such as basic recombinant DNA technology for the cloning, propagation, and sequencing of recombinant plasmids, and preparation of RNA and analysis by RNA Northern blots. Immunohistochemical staining techniques were used to detect TGF-β proteins. Sandwich enzyme linked immunosorption assays (SELISA) were used to quantitate the levels of TGF-β1 and TGF-β2 proteins. Colony formation was performed using soft agar growth assays.

Major Findings:

To investigate the function of TGF-β and its potential use as a therapeutic agent, we studied the expression of TGF-β1, 2 and 3 mRNAs in cultured NSCLC and SCLC cells. A survey of 12 NSCLC cells including representative adenocarcinoma, squamous cell carcinoma, large cell carcinoma, carcinoid, and branchioalveolar carcinoma cells and 6 SCLC cells including classic and variant cells was conducted. Northern blot analysis of RNA from these cells showed expression of TGF-β1, 2 and 3 mRNAs in both NSCLC and NSCLC cells, with the level of expression of each mRNA being higher in NSCLC than in SCLC cells. The relative abundance was TGF-β1 mRNA > TGF-β2 mRNA ≥ TGF-β mRNA. Northern blot analysis of these cells also showed expression of TGF-β type II and TGF-β type III receptor mRNAs, with the level of expression of each mRNA being higher in NSCLC than in SCLC cells. Expression of TGF-β type II receptor mRNA was significantly higher than that of TGF-β type III receptor mRNA in NSCLC cells.

Northern blot analysis of NSCLC and SCLC cells showed expression of RAR and RXR mRNAs. Expression of RAR-α, RAR-β and RAR-γ mRNAs was approximately equal in NSCLC cells, while expression of RAR-α mRNA was equal to or greater than that of RAR-β mRNA in SCLC cells. Expression of RAR-γ mRNA was less than that of both RAR-α and RAR-β mRNAs in SCLC cells. In addition, expression of RXR-α, RXR-β and RXR-γ mRNAs was approximately equal in both NSCLC and SCLC cells, with expression of RXR-γ > RXR-β > RXR-α in both cell types. Four NSCLC cells expressing different levels of TGF-β mRNAs were chosen to investigate responsiveness to retinoic acid. These included the squamous cell carcinoma NCI-H157, the carcinoid NCI-H727, the adenocarcinoma NCI-H838 and the large cell carcinoma NCI-H1299. Each of these cells was incubated with 1 ng/ml of all-*trans*-retinoic acid for 24 hours and examined for expression of TGF-βs 1, 2 and 3 mRNAs using specific cDNA probes for these isoforms. Northern blot analysis showed a significant increase in the level of expression of TGF-β mRNA in H727 cells, while expression of the mRNAs for TGF-βs 2 and 3 were not changed upon treatment with retinoic acid. In contrast, in H838 cells, expression of TGF-β2 mRNA increased after incubation with retinoic acid, while expression of TGF-βs 1 and 3 mRNAs was not affected. Furthermore, in H1299 cells, treatment with retinoic acid did not change expression of any of the TGF-β isoforms.

To investigate possible TGF-β isoform-specific effects upon treatment with retinoic acid, immunohistochemical staining patterns of the TGF-β isoforms were examined in those NSCLC cells whose TGF-β mRNA expression was affected by treatment with retinoic acid. For these studies, TGF-β affinity-purified peptide antibodies that had previously been demonstrated to be specific for each isoform were used. Immunostaining of H727 cells with the TGF-β antibodies showed increased staining of TGF-β1 after treatment with retinoic acid, while the patterns of staining for TGF-βs 2 and 3 were not changed in these cells. Immunostaining of H838 cells with these antibodies showed increased staining of TGF-β2 after treatment with retinoic acid, while the patterns of staining for TGF-βs 1 and 3 were not changed in these cells. The pattern of immunostaining for TGF-βs 1, 2 and 3 in H1299 cells, was not affected by treatment with retinoic acid.

To examine the ability of retinoic acid to control cell proliferation, H727 and H838 cells were incubated with 1 ng/ml of all-*trans*-retinoic acid in soft agar for 2 weeks. Both the number and size of the colonies were inhibited by treatment with all-*trans*-retinoic acid. When these cells were incubated similarly with 9-*cis*-retinoic acid and 13-*cis*-retinoic acid, the number and size of the colonies were also inhibited, but inhibition in the presence of all-*trans*-retinoic acid was greater than in the presence of either 9-*cis*-retinoic acid or 13-*cis*-retinoic acid.

We have demonstrated expression of TGF-β1, 2 and 3 mRNAs in both NSCLC and SCLC cells and coordinate expression of the 3 TGF-β proteins in NSCLC cells. In addition, we have shown expression of TGF-β type II and TGF-β type III receptor mRNAs. We have also shown

expression of RAR and RXR mRNAs in NSCLC and SCLC cells. Furthermore, we have shown in some NSCLC cells that TGF-β expression is differentially affected by treatment with retinoic acid at both the mRNA and protein levels. We have also shown that growth of some NSCLC cells in soft agar could be inhibited after incubation with all-*trans*-retinoic acid.

Cellular Differentiation in Normal and Neoplastic Respiratory Epithelium
(Z01 CN 00167-02 BPRB)

The incidence of lung cancer in the United States continues to increase, and there are over 170,000 new cases predicted for 1993. Presently lung cancer is the leading cause of cancer deaths in both men and women. The biology of normal and neoplastic lung is complicated by the fact that the cells of respiratory epithelium can differentiate along multiple pathways that have neoplastic correlates with distinct clinicopathologic features. Our goal is to explore cellular differentiation at the molecular level to give rational basis for prevention, diagnosis, and treatment of lung cancer. This has been studied at the level of 1) neuroendocrine differentiation, 2) peripheral airway cell differentiation, 3) Clara cell specific protein, and 4) oncogene expression.

Materials and Methods:

Panels of well characterized human lung cancer cell lines of all histologic types with detailed clinical information were used. In addition, normal tissue and newly diagnosed, resected lung cancers with surrounding non-neoplastic lung were utilized. Sensitive immunohistochemical techniques with avidin biotinylated peroxidase and RNA-RNA in situ hybridization with autoradiography were used to localize differentiation markers and to study the expression of oncogenes at the protein and messenger RNA level.

1) Neuroendocrine differentiation. Small cell lung cancer (SCLC), characterized by neuroendocrine (NE) differentiation, is sensitive for cytotoxic therapy. We have demonstrated that 15% of non-small cell lung carcinomas (NSCLC) also express multiple NE features. Our results indicate that these tumors are initially sensitive to chemotherapy. The role of NE differentiation in non-neoplastic epithelium is being investigated.

2) Peripheral airway cell differentiation. The incidence of adenocarcinoma in the United States is increasing. In our experience up to 50% of adenocarcinomas may show marked areas with bronchioloalveolar or papillary growth pattern. The progenitor cells for these tumors, as well as peripheral airways epithelium, include Clara cells and type II pneumocytes, which are characterized by their defined products CC10 and SP-A, respectively. We found 30% of the 400 NSCLC tumors examined to be positive for at least one of the peripheral airway cell (PAC) markers CC10 and SP-A. Clinically, these tumors were associated with younger age and lighter smoking history. Characterization of NSCLC cell lines expressing PAC markers is in progress. In order to examine premalignant lesions of peripheral lung cancers which are currently poorly understood, we exposed Syrian golden hamsters to NNK, a tobacco-specific nitrosamine, which is a systemic carcinogen in these animals. The respiratory epithelium in hamsters closely resembles that of man. In a serial sacrifice study, 100% of the animals had lung tumors at 24 weeks. Lungs and serum at 2-24 weeks are currently examined for premalignant changes and peripheral airway and neuroendocrine cell differentiation.

3) Clara cell specific protein (CC10). We have demonstrated that nonciliated secretory cells, which are progenitor cells for the epithelium and NSCLC, express high levels of CC10, while only 10% of NSCLC are positive for CC10. Preliminary studies have shown that the levels of CC10, which is also known as PCB (a potent carcinogen)-binding protein, are affected by exposure to carcinogens. In human lung immunohistochemistry revealed CC10 reactivity in the cytoplasm of nonciliated secretory cells throughout the conducting airways. Epithelial basal cells, and alveolar cells were negative. The expression of mRNA paralleled the expression of

protein. Most abundant mRNA was seen in bronchioli. In the presence of morphologic atypia, CC10 expression decreased in bronchi, and became detectable in up to 20% of alveoli. Changes were minimal in bronchioli. The extent of morphologic atypia correlated with smoking history.

4) Oncogene expression. Using human lung cancer cell lines we have previously demonstrated that p53 (a suppressor oncogene) immunoreactivity correlates with the class of mutations. Since the major function of p53 is in controlling cell proliferation, we studied the prognostic impact of p53 overexpression and the expression of proliferating cell nuclear antigen (PCNA) in a well characterized cohort of 120 NSCLC patients. For a subset of patients in a potentially curative group both p53 and PCNA were predictive of shorter survival in a univariate analysis. In a multivariate analysis only p53 was a significant prognostic factor. Molecular studies of the corresponding mutations are in progress. In the surrounding non-neoplastic lung clusters of peripheral airway cells were frequently positive for markers of proliferating cells (PCNA and Ki67), overexpressed *c-myc*, but lacked p53 immunoreactivity. However, p53 was prominent in the lesions of more central airways.

The significance of the project:

The results of these studies will provide a rational basis for innovative approaches for early detection and intervention in human lung cancer. The information of premalignant changes preceding SCLC or adenocarcinoma are not known. Differentiation markers are necessary tools to reveal the appropriate cell populations in the epithelium that are targets for the promotional events leading to lung cancer, and should also become targets for early intervention.

***CYP1A1* Gene Regulation and Human Cancer** (Z01 CN 00168-02 BPRB)

The *CYP1A1* isoenzyme is a member of the cytochrome P450 superfamily. The *CYP1A1* gene product, aromatic hydrocarbon hydroxylase (AHH), is involved in the metabolic activation of pulmonary procarcinogens and can be induced by polycyclic aromatic hydrocarbon components of tobacco smoke condensate. Individuals with elevated levels of AHH may be at an increased risk of developing lung or other tumors.

A preliminary study conducted by McLemore *et al.* revealed that 60% of human non-small cell lung cancer (NSCLC) cell lines had abnormally high basal (non-induced) levels of expression of the *CYP1A1* gene and of AHH enzymatic activity. This prompted us to investigate the regulation of *CYP1A1* in normal and neoplastic lung. We are using a large panel of well characterized human lung cancer derived cell lines to study the mechanisms involved in the regulation of expression of the *CYP1A1* gene and to identify the genetic alterations that may have resulted in the elevated levels of *CYP1A1* gene products.

The significance of the project is to determine, for the first time, the interactive role of genetically determined factors and chemical carcinogens in pulmonary carcinogenesis. The results should have important diagnostic and prevention applications.

The expression of human *CYP1A1* and its potentially significant role in tumorigenesis was analyzed at the levels of 1) general regulatory patterns, 2) activator-repressor interactions, 3) feedback modulation via end product(s), and 4) proto-oncogene interactions.

1) General regulatory patterns. We have conducted detailed analyses of the patterns of expression of human *CYP1A1*. Oligonucleotide directed mutagenesis (ODM) has been carried out on a number of DNA sequence elements within the regulatory region of the human *CYP1A1* gene. These altered regulatory sequences were then fused to the bacterial reporter gene chloramphenicol acetyltransferase (CAT).

Through the introduction, via stable transfection, of numerous such constructs into several non-small cell lung cancer derived lines, followed by measurement of the amounts of the CAT gene product, we have identified a unique mechanism for the expression of the *CYP1A1* gene. Our results indicate that two transcriptional activation elements nearly five hundred base-pairs apart are responsible for the expression of the gene. The two elements are the interaction sites for the aromatic hydrocarbon receptor. Control of *CYP1A1* expression from the two sites appears to be additive with expression from each contributing roughly 50% of the total activity.

To differentiate *cis* versus *trans* effects on the expression of the *CYP1A1* gene, we have succeeded in the cloning of portions of the *CYP1A1* gene regulatory region from several of the NSCLC derived lines used in these studies. These regulatory regions are being characterized further by inserting the regions of interest into several reporter gene vectors. These constructs will then be introduced into a number of cell lines and the patterns of expression observed.

2) Activator-repressor interactions. Oligonucleotide directed mutagenesis of DNA sequence elements within the regulatory region of the *CYP1A1* gene have provided a great deal of insight into the motifs that, upon interaction with the appropriate DNA-binding protein(s), result in transcriptional activation of the *CYP1A1* gene. One model of the regulation of *CYP1A1* gene expression incorporates a constitutive transcriptional repressor protein. Evidence for the existence of such a regulatory protein arises from several different studies. Altered interaction of a repressor protein may, in some cases, be the cause of elevated levels of *CYP1A1* expression. This is currently under investigation.

3) Feedback modulation of expression. In addition to the existence of a potential negative transcriptional regulatory protein, results of previous studies by others working with murine hepatoma derived cell lines suggest that the *CYP1A1* gene product aromatic hydrocarbon hydroxylase (AHH) may play a significant role in regulating its own expression by an, as yet, undetermined mechanism.

We are currently investigating the possibility that the elevated basal levels of fully functional AHH protein observed in the non-small cell lung cancer derived cell lines arise as a result of one or more mutations occurring within the coding sequence of the human *CYP1A1* gene.

We are in the process of cloning the *CYP1A1* cDNAs from a large battery of non-small cell lung cancer derived cell lines known to express high basal levels of AHH. cDNA from tumor derived material shown to express *CYP1A1* through in situ hybridization or immunohistochemical techniques will also be obtained. These clones are to be introduced into a series of *CYP1A1* null mutation cell lines that are being created expressly for this purpose. Use of the *CYP1A1* null mutants will permit an in depth study of protein function in the absence of the endogenously expressed gene. Those clones demonstrating aberrant expression patterns will be analyzed further.

4) Proto-oncogene interaction. Some preliminary studies indicate a possible role for the proto-oncogenes *c-jun* and *c-fos* in the regulation of expression of the *CYP1A1* gene. Studies using oligonucleotide directed mutagenesis, gel-shift analysis, and synthetic constructs are being carried out to define the role(s) of *c-jun* and *c-fos* in *CYP1A1* gene expression.

Biological Regulation of Lung Cancer Growth (Z01 CN 00171-02 BPRB)

The purpose of this project is to develop new rational agents to intervene in the important biochemical pathways of early cancer cells. Ultimately, a successful intervention agent will be administered chronically to large populations of healthy subjects. One approach to minimizing the toxicity of such a strategy is to use an agent with a very selective mechanism of action. Through systematic study of the biology of early cancer cells, we are attempting to identify such mechanisms.

The addition of Dr. Terry Moody to the BPRB staff allows the Branch to expand the range of its growth factor biology studies. Dr. Moody is one of the world's leading authorities on the biology of neuropeptide receptor activation, especially as it relates to growth stimulation. Recent findings with the use of vasoactive intestinal peptide antagonists suggest applications of the type of antagonist for general application in the regulation of neuropeptide mediated cancer growth (Z01 CN 00182-01 BPRB). Likewise the family of transforming growth factor Beta provides another class of mediators with relevance for regulating not only breast cancer but also lung cancer growth (Z01 CN 00166-02 BPRB).

The methods used for this research include a variety of proliferation assays including clonogenic assay, semi-automated colorimetric assay, thymidine uptake, and a variety of column chromatographic procedures for the isolation of mediators of intracellular signal transduction.

We have evaluated a number of compounds that influence the growth of lung cancer cells. We have reported previously on the autocrine role of gastrin releasing peptide, insulin-like growth factor, and transferrin—all of which stimulate growth factor for certain types of lung cancer. We have also shown that regulatory molecules such as glucagon and 13-*cis*-retinoic acid can inhibit the growth of a number of lung cancer cells lines. This experience has allowed us to focus on the signal transduction pathways most central to the process of cellular proliferation. In collaboration with Dr. M. Jett, we have recently presented data suggesting that 5-HETE, a product of 5-lipoxygenase activation, may be a key intermediary in growth factor mediated growth stimulation of cancer cells. Since considerable information exists about the lipoxygenase pathway, we can potentially exploit the availability of existence of specific antagonists for application as biointervention tools. Systematic evaluation of the growth factor biology of early cancer cells may yield additional clues for the development of rational cancer intervention agents.

The most interesting *in vitro* leads will be evaluated for clinical application in Phase I and II studies conducted by the BPRB. An important part of that effort would be the identification of markers for intermediate end points analysis; these would accelerate the process of determining the benefit of this class of intervention tools.

Biochemistry of Peptides and Growth Factors in Lung Cancer (Z01 CN 00182-01 BPRB)

Objectives

This project investigates peptides and growth factors in lung cancer. Specifically the ability of peptide receptor antagonists and monoclonal antibodies to inhibit lung cancer proliferation is determined.

Methods

Lung cancer cells and hybridomas are grown using cell biology techniques. Peptides and growth factors are identified by Northern blot analysis and radioimmunoassay. Receptors are identified by Northern blot analysis and receptor binding assays. Second messengers are identified using radioimmunoassay, fluorescence and Western blot techniques. Growth assays are performed *in vitro* and *in vivo*.

Major findings

Previously we found that GRP was an autocrine growth factor in some SCLC cell lines. In the current year, the gene expression of GRP was investigated. Forskolin increased GRP gene expression in NCI-H209 cells 3-fold after 8 hours. Forskolin elevated the cAMP 10-fold and increased *c-fos* gene expression in SCLC cells. Cyclic AMP activates protein kinase A resulting in the phosphorylation of protein substrates. The protein substrates may interact with the 5′ regulatory region of the GRP gene stimulating transcription.

GRP receptors were solubilized in a high affinity ligand binding conformation using CHAPS/cholesterol hemisuccinate. The GRP receptors were purified 86,000-fold using affinity chromatography techniques. A major 65 Kdalton band was purified. The GRP receptor is a 384 amino acid protein comprised of 384 amino acid residues and 7 hydrophobic domains. The solubilized and purified GRP receptor may prove useful for reconstitution studies.

The neuromedin B (NMB) receptor has 56% homology to the GRP receptor. We found that (^{125}I-Tyr^{0})NMB binds with high affinity to several SCLC cells and specific (^{125}I-Tyr^{0})NMB binding is inhibited with high affinity by NMB and moderate affinity by GRP or bombesin (BN). GRP receptor antagonists such as (D-FPhe^{6}, D-Ala^{11})BN$^{6-13}$ methyl ester ((FA)BN$^{6-13}$ME) only weakly inhibited specific (^{125}I-Tyr^{0})NMB binding. NMB elevated the cytosolic Ca^{+2} in Fura-2 AM loaded NCI-H345 cells and the ability of NMB to elevate cytosolic Ca^{+2} or stimulate clonal growth was only weakly antagonized by (FA)BN$^{6-13}$ME. NMB was antagonized, however, by (D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}) substance P. These data suggest that distinct GRP and NMB receptors are present on SCLC cells.

Continued progress was made on GRP receptor antagonists. (FA)BN$^{6-13}$ME weakly inhibited xenograft formation in nude mice. In contrast, (Psi^{13,14}, Leu^{14})BN (10 μg/day, subcutaneous) strongly inhibited xenograft formation in nude mice. (FA)BN$^{6-13}$ME was only slowly metabolized *in vivo*. Current efforts focus on the biodistribution of GRP receptor antagonists using nude mice containing SCLC xenografts. GRP receptor antagonists may prove useful as SCLC therapeutic agents.

High levels of vasoactive intestinal polypeptide (VIP) mRNA were detected in NSCLC cells by Northern blot analysis. Unfortunately only low levels of VIP were detected by radioimmunoassay. Recent data indicate that NSCLC cell extracts and conditioned media have higher levels of proVIP than VIP. These data suggest that VIP does not readily get processed in NSCLC cells. ProVIP was demonstrated, however, to have appreciable biological activity.

A new VIP receptor antagonist, VIPhybrid was identified, which inhibits the proliferation of lung cancer cells. VIPhybrid contains the N-terminal 6 amino acid residues of neurotensin and the C-terminal 22 amino acid residues of VIP. Specific ^{125}I-VIP binding was inhibited with high affinity by PACAP (IC_{50} = 10 nM) and moderate affinity by VIPhybrid (IC_{50} = 700 nM). VIP and PACAP elevated the cAMP whereas VIPhybrid inhibited the increase in cAMP caused by VIP. VIP stimulated colony formation whereas VIPhybrid inhibited the increase in colonies caused by VIP. VIPhybrid inhibited basal colony formation and lung cancer xenograft formation in nude mice. Because both SCLC and NSCLC have VIP receptors, VIPhybrid may be a useful peptide to inhibit lung cancer growth.

Also, lung cancer cells have distinct PACAP receptors. Specific ^{125}I-PACAP binding was inhibited with high affinity by PACAP but moderate affinity by VIP. Also, PACAP elevated cytosolic Ca^{+2} in Fura-2AM loaded NCI-H345 cells whereas VIP had no effect. These data suggest that lung cancer cells have distinct VIP and PACAP receptors.

Immunocytomorphic Test for Early Lung Cancer Detection (Z01 CN 00172-02 BPRB)

The purpose of this project is to develop new tools for lung cancer early detection. The first aspect is the confirmatory trial of sputum immunostaining as an early detection tool. We propose that the epithelium is the appropriate target for early detection of lung cancer and that monoclonal antibodies allow detection of the over-expression of certain classes of molecules involved in the process of neoplasia.

The methods used in this effort include immunocytochemistry and image analysis (to quantitate assay end points). A variety of immunologic and enzymatic assays are being used to quantitate the growth factor expression in the bronchial lavage.

We have established a CRADA (#35516) to prospectively validate the diagnostic accuracy of a lung cancer early detection approach. A clinical team of investigators from 11 institutions throughout the United States and Canada is accruing stage I resected lung cancer patients to a protocol where annual induced sputums will be acquired and immunostained. Ongoing patient followup will permit the eventual correlation of immunostaining status with clinical outcome (correlation of positive immunostaining with the development of lung cancer and vice versa). Immunostaining for this study will be done at the University of Pennsylvania, and data acquisition and analysis will be handled at Johns Hopkins University. As part of this effort, selected patients will undergo bronchoscopy, and their bronchial lavage fluids will be studied for the quantity and quality of growth factor expression. We have developed a variety of methods for assessing the proliferative capacity of bronchial lavage products in an effort to complement the sputum immunocytology approach in determining who is and is not at risk for manifesting lung cancer. The details of the bronchial lavage assay methodology have been recently published.

An archive of sputum and other clinical specimens remaining after the primary analysis will be conserved to permit rapid analysis of other new promising early detection markers. All CRADA funds are being expended to support the clinical trial, and none of these funds are being spent at the NCI.

The BPRB is diversifying its efforts to begin systematic development of new early diagnostic tools for breast and female reproductive cancers. As with lung cancer, molecular and cellular changes which occur on the carcinogen-transformed epithelial surface of the relevant organs will define attractive targets for evaluation as early detection tools. Branch publications from the proposed Molecular Mechanisms Section are already identifying the relevant targets for further consideration as early detection tools.

Core analysis includes quantitation of autocrine growth factors such as GRP as well as more global assessment of neuroendocrine activation by monitoring levels of peptidyl amidating monooxygenase (PAM) activity. This application builds upon the biology elucidated in our lab, establishing the role of this enzyme system in contributing to the chronic growth stimulation of neoplastic pulmonary epithelium. This work has major relevance in developing new early lung cancer detection approaches.

<u>Evaluation of Markers for the Early Detection of Lung Cancer</u> (Z01 CN 00183-01 BPRB)

The early detection of lung cancer is critical to improving the mortality rate associated with lung cancer. Studies from the Lung Cancer Study Group have shown that the 5-year survival of patients with very early stage non-small cell lung cancer (T1N0M0) is 80%, far better than the overall 10% 5-year survival for lung cancer. We have had an ongoing collaboration with Dean Cole at the Los Alamos National Laboratory to evaluate a new photoactive porphyrin compound for the detection of early lung neoplasms, and use a radiolabeled form of this compound for local ablation of abnormal bronchial epithelium (in project Z01 CN 00169-02 BPRB). This project is awaiting further development of the porphyrin compound.

During the summer of 1993, protocols for the early detection of lung cancer among individuals at high risk will be submitted for approval. We propose to target lung cancer and head and neck cancer survivors with serial monitoring of sputum and bronchoscopically obtained specimens to assay for biomarker expression. The study design will incorporate comparison of findings in different specimens such as bronchial washings, bronchial biopsies at multiple sites and expectorated sputum. Particularly important would be whether or not the markers are differentially expressed in the unaffected portions of the lung or only detectable in certain types of specimens.

A subset of subjects will be enrolled in an intervention trial. Specimens will be obtained at on-study, after the period of intervention and roughly 3 months after the intervention is stopped. The first intervention trial anticipated will use 4-HPR if available, or low dose 13-*cis*-retinoic acid. Future intervention agents for this subject population might include Vitamin E or the radio-labeled photoactive porphyrin compound for a 3-6 month period. Pharmacokinetic studies will also be incorporated to evaluate the effect of dose on marker modulation as well as to evaluate alternative forms of administration such as aerosolization of the agents. The success of early detection of cancer is dependent upon the ability to intervene successfully at that early stage to prevent the morbidity and mortality associated with cancer and standard therapy.

Published reports have suggested that genomic p53 mutations may be present in individuals with an inherited susceptibility to several types of cancer, including but not limited to Li-Fraumeni syndrome. A similar study using genomic DNA from whole blood collected during a case-control study of lung cancer is planned to evaluate markers of susceptibility to lung cancer.

Rational Applications of Biomarkers in Clinical Trials (Z01 CN 00169-02 BPRB)

A major challenge for the BPRB is to begin to apply specific biomarkers in a rational way to permit more effective early detection approaches. Our laboratory resources will permit an intensive characterization of biomarkers and the biologic effects of intervention agents in a pilot study setting. Multiple markers have been implicated in the pathogenesis of human malignancies. Point mutations in the *ras* oncogene have been identified in colorectal and lung carcinomas and have recently been identified in shed epithelial cells found in stool specimens from patients with colorectal carcinomas. Alterations in carbohydrate antigen expression have also been found in malignancies and may be useful markers of neoplastic change.

Shed epithelial cells in archived stool specimens from patients with documented colorectal cancer will be analyzed using polymerase chain reaction for oncogene mutations or activation, or changes in carbohydrate antigen expression. These findings will be correlated with the markers present in the archived surgical material. Blocks from patients entered on a case-control study of colorectal cancer conducted at the National Naval Medical Center, Bethesda, Maryland have been obtained and are being analyzed for the presence of *ras* mutations and p53 expression. They will also be examined for carbohydrate antigen expression. If preliminary results are promising, additional specimens from patients entered on this study at Walter Reed Army Hospital and George Washington University Hospital, Washington, DC will also be obtained. Assays on stool specimens from control subjects will also be performed to assess the usefulness of these markers to discriminate patients with colorectal cancer from controls without cancer. The information obtained will be coupled with the previously collected epidemiologic data and Tumor Registry data for survival information to determine the potential usefulness for screening or prognostic purposes.

Clinical Evaluation of New Intervention Agents (Z01 CN 00170-02 BPRB)

The purpose of this project is to evaluate if autocrine growth factors play a major role in the period of cancer promotion and whether they comprise a class of molecules that are appropriate targets for cancer intervention approaches.

These experiment growth rate assays involve a range of proliferation assays, *in vivo* hetero-transplant assays, receptor assays, functional assays and pharmacokinetic analysis.

We and others have demonstrated the role of gastrin releasing peptides (GRP) as an autocrine growth factor, and the weight of this evidence is consistent with GRP playing an important role in early cancer formation. We have evaluated the use of a neutralizing mono-clonal antibody to block the effect of this growth factor in patients with advanced small cell lung cancer. This treatment is associated with no demonstrable toxicity, but only one patient had a significant anti-tumor response.

Based on this experience, we proposed to evaluate a new class of GRP antagonists that are synthetic peptides. This class of molecules may have better properties such as bioavailability and affinity than monoclonal antibodies. These molecules might also be tagged with a radioisotope to permit exact pharmacologic analysis. Synthetic peptide growth factor antagonists may be very useful for delivery as intervention agents, and we proposed to evaluate that possibility. This effort would be a model for the type of rational intervention agent research that the BPRB staff will conduct.

Several peptide antagonists have been identified by evaluating *in vitro* cytotoxicity with lung cancer cell lines. These same analogues have been shown to have consistent growth inhibitory effects *in vivo* with mouse xenografts. Prior to evaluation in humans, acute and chronic toxicology studies must be performed to evaluate for unexpected patterns of side effects. We have already devised an approach to optimal dose determination for anti-GRP monoclonal antibodies; we can extend this analysis to the peptide antagonists to determine the validity of this approach in a new system.

GRP is a molecule that serves as an excellent model of a neuropeptide effector that may be important as a mediator of tumor promotion dynamics in certain epithelium; as such it comprises an attractive target for intervention research.

Identification of Peptide Growth Factors That Regulate Human Tumor Proliferation
(Z01 CN 00173-02 BPRB)

All bioactive peptides are originally derived from larger precursor molecules. Post-translational processing events are responsible for excising the peptide from its respective prohormone and conveying biological integrity to the liberated ligand. One of the few post-translational events that tracks exclusively with bioactivity is α-amidation. By defining the amino acid motifs that function as signals of α-amidation, one can predict the formation of biologically relevant peptides hidden within the preproprotein. We have applied this research strategy to analyze the precursor proteins of previously cloned human growth factors. These include atrial natriuretic factor (ANF), epidermal growth factor (EGF), endothelin-1 (ET-1), gastrin-releasing peptide (GRP), insulin-like growth factors I and II (IGF-I and IGF-II), transferrin (TF), and transforming growth factor α and β (TGF-α and TGF-β). Without exception, we have identified amino acid consensus sequences within these precursors molecules that predict the formation of additional bioactive peptides. For example, human EGF is composed of 53 amino acids residues (a.a.) and is generated from a precursor protein of 1,207 a.a. in length. Located amino-flanking to EGF are eleven separate and distinct amidation motifs that implicate alternative peptide products.

In those instances where alternative mRNA processing gives rise to two precursor proteins (i.e., IGF-IA and IGF-IB), it is the longer precursor only that shows selective expression of amidation motifs. In the proform of IGF-IA (105 a.a.) there are no amidation sequences present, however, in the prohormone of IGF-IB (147 a.a.) there are at least two putative amidation sequences. Thus alternative mRNA processing may represent a level of control the cell uses to regulate peptide amidation expression. As an extension of this initial discovery, we began to examine if synthetic peptide amides, derived from the putative a.a. sequences encoded in the prohormone, had growth promoting effects on normal and malignant pulmonary cells.

We have identified two putative amidation motifs located within the E domain of proIGF-IB (C-flanking from IGF-I peptide). The first peptide amide, IBE_1, with a.a. sequence of G-W-P-K-T-H-P-G-G-E-Q-K-E-G-T-E-A-S-L-Q-I-R-NH_2, is assigned to region $IGF\text{-}IB_{103\text{-}124}$. The second peptide amide, IBE_2, is located at $IGF\text{-}IB_{129\text{-}142}$ and has an a.a. sequence of E-Q-R-R-E-I-G-S-R-N-A-E-C-R-NH_2. Synthetic peptide homologs were generated to the predicted sequence as the tyrosine zero derivative to allow for radioactive labeling with ^{125}I isotope.

The IBE_1 peptide amide was found to stimulate both normal and malignant pulmonary cells over a dose range of 10-100 nM. The peptide's growth effects were shown to be dependent on an intact carboxy-terminal amide (free-acid derivative was 100-1000 x less potent). In receptor binding studies with ^{125}I-IBE_1 we have determined that the peptide mediates its growth promoting effects through a unique receptor not related to the type I receptor of IGF-I, nor to the type II receptor of IGF-II, nor to the insulin receptor. In addition, a neutralizing monoclonal antibody to the type I receptor, aIR3, which blocks IGF-I growth stimulation, did not inhibit the trophic effects of IBE_1. Scatchard analysis of IBE_1 receptor expression on A549 (bronchioalveolar CA) revealed a K_D 2.8×10^{-11} and density of 1.5×10^4 per cell.

High titered rabbit polyclonal antisera were generated to the synthetic homolog of IBE_1 and were used for the immuno blot analysis of several normal and malignant tissue. Immunoreactive IBE_1-like peptides were identified in two small cell lung cancer cell lines and have molecular weights of 25 kDa, 12 kDa, 8 kDa, and 5 kDa. In addition, similar immunoreactive peptides have been identified in normal liver extracts from a variety of mammalian species, implicating evolutionary conservation.

Preliminary studies with IBE_2 peptide have indicated a selective growth promoting effect for several human colon cancer cell lines.

Future research studies will include the chemical characterization of isolated peptide products (isoelectric focusing and high performance liquid chromatography purification schemes) that express common immuno epitopes to IBE_1. This will include determination of total a.a. composition (triple "A" analysis) and a.a. sequence of isolates, and mass spectrometric analysis. The studies will offer definitive proof that the putative peptide amides predicted to be formed from proIGF-IB have physiological relevance in both normal and malignant environments. These peptides also represent rational biological targets for the early diagnosis and prevention of human pulmonary cancers.

Identification of Peptide Growth Factor Binding Proteins (Z01 CN 00174-02 BPRB)

There are several mechanisms by which the cell can modulate peptide induced growth effects. Traditionally, this has been encompassed by transcriptional and translational events. However, there are several alternative levels of control that the cell utilizes to regulate proliferation. These include post-translational modifications that convey biological integrity to the peptide growth factor, active secretion that release the peptide into the external milieu, functional receptor expression that allow biological ligand interaction, activation of secondary signal transduction pathways (i.e., Ca^{+2} flux, PI turnover, cyclic nucleotide concentration, etc.) that serve as an internal communication network to the nucleus, naturally occurring binding proteins that regulate ligand/receptor availability, and proteolytic enzymes that degrade the peptide and abolish bioactivity.

Recent evidence has demonstrated that a variety of human peptide growth factors have associated with their function naturally occurring binding proteins (BP) which modulate the ligand mediated proliferative response. The best example of this interaction is seen with insulin-like growth factor I (IGF-I) and at least five distinct BPs (encoded on different genes) that regulate IGF-I/receptor binding. It has been previously shown that different IGF-I BPs can have dichotomous effects on IGF-I induced growth. Some BPs bind to IGF-I, causing alterations in the ligand's structural conformation that results in enhancing type I receptor affinity and augmenting IGF-I's proliferative effect; these are superagonists. In contrast, other BPs interact with IGF-I to induce a steric interference of type I receptor binding and thereby block IGF-I effects; these are antagonists.

We have begun to investigate if there exist naturally occurring BPs for the mitogenic peptide amide IBE_1 that may function as biological regulators of growth. Towards this end, we examined if plasma or serum from normal and diseased individuals (lung cancer patients) contained such entities. Initial screening studies utilized ^{125}I-IBE_1 as a labeled tracer to determine the presence of BPs. Plasma or serum sample were incubated with the labeled ligand over different time courses (2 hr, 4 hr, 24 hr) and assessed for the presence of BPs by agarose electrophoresis. Alterations in the electrophoretic mobility (EM) of the free ligand over the test samples wereinterpreted as a positive indicator of BP expression. In normal individuals there were dramatic differences in the electrophoretic profile observed between homologous plasma/serum samples. Plasma universally showed no altered EM shifts over the free-ligand. However, the sera of all 6 normal donors demonstrated the existence of at least three distinct BPs having EMs of 1.4 cm, 2.6 cm, and 4.2 cm from the origin. Differences in the binding patterns observed between plasma and serum were not due to the presence of a chelator since NaCitrate at 1x, 2x, and 10x concentration used to generate the plasma did not alter serum binding results.

The IBE_1/BP complexes were pH sensitive and dissociated under acidic conditions (pH 2.0). This physical phenomenon was used to verify that the observed binding patterns of serum were not just a result of enzymatically degraded tracer. Following a 2-hour exposure of pooled normal serum with labeled tracer, the sample was subjected to high performance liquid chromatography (HPLC) and assessed for ligand integrity. It was shown that although three BPs were detectable by electrophoretic analysis, only one peak of labeled tracer (intact) was eluted from the sample by HPLC under acidic conditions (0.1% TFA) with a gradient of acetonitrile (15-30% CH3CN).

We are now beginning to determine if these observed BPs are specific for IBE_1 binding. This will be accomplished using cold ligand (2μM) to compete for ^{125}I-IBE_1/BP interaction. Several peptides (IBE_1, IBE_2, IGF-I, IGF-II, insulin, GRP, substance P, VIP, and GPL-I) will be used for this study to confirm the mode of binding. If specific binding is taking place, it is assumed that only cold IBE_1 is as effective in blocking BP interaction as is the amide counterpart. In addition, we have started to characterize the BPs in question by immunological means using cross-immunoelectrophoresis. Finally, BPs will be examined for their ability to regulate the mitogenic effects of IBE_1 on normal and malignant pulmonary cells. These BPs potentially represent naturally occurring biologics that could be incorporated into the early detection and prevention of malignant disease

Post-Translational Processing Mechanisms in Tumor Cells (Z01 CN 00175-02 BPRB)

Objectives

This project is directed at identifying biochemical mechanisms of tumor cells which may be targets for early detection, intervention and/or therapeutic strategies, with a focus on the enzymatic processes responsible for post-translational processing of inactive precursor prehormones into bioactive peptide hormones.

Our studies of tumor cell enzymes required for processing of precursor prehormones to active peptide hormones are comprised of three parts:

A: Biochemistry of peptidyl amidating enzyme complex
B: Molecular biology of endo- and exo-protease enzymes
C: Effect of inhibiting peptidyl amidating enzyme on cell growth

Methods employed and major-findings

Part A of this study is principally carried out using a variety of protein chemistry techniques. The two enzymes responsible for peptide amidation (PHM and PGL) are synthesized from the same gene and mRNA precursor, and are cleaved to two separate enzyme activities.

We developed assays for each of these enzymes using a small (tripeptide) substrate. We have identified several different molecular forms of both PHM and PGL in lung tumor cell lines. Our studies on the biochemistry of the different molecular forms of the enzymes is continuing. We are purifying two proteins with PHM activity and one bifunctional PHM+PGL enzyme. We are also developing pcr-based techniques to enable us to differentiate the forms of these enzymes at the mRNA level. We have recently found that a large number of non-endocrine lung tumor cell lines, and many non-lung tumor cell lines, express PAM mRNA. We are characterizing these non-lung enzymes biochemically. Our preliminary work in non-endocrine lung cell lines shows disparate expression of PAM gene and other genes of the endocrine phenotype, such as DOPA decarboxylase.

As described in the last report, we are carrying out part B of this study at the level of mRNA expression. So far, only a few tumor cell lines have been identified which express different members of the endoprotease enzymes in amounts sufficient for detection by Northern blot analysis. We are analyzing cell lines which have undetectable levels of expression by the more sensitive technique of reverse transcriptase-polymerase chain reaction (rt-pcr). As the prehormone processing endoproteases are members of a fairly highly conserved family of enzymes, we are obtaining nucleotide sequence for the cDNAs obtained by rt-pcr to confirm their identity.

Several of the cell lines which express peptidyl amidation enzymes have been reported to be dependent on autocrine (self-stimulatory) loops involving amidated peptides such as GRP. We have used growth assays to explore whether inhibition of the amidating enzymes will result in growth inhibition of these cell lines (Part C). Two classes of compounds which we have shown to be inhibitors of human tumor PHM in cell extracts have been used. Inhibitors which chelate copper, an essential metal ion cofactor of PHM, do inhibit cell growth in cell lines expressing high levels of PHM activity, and may have less effect in lines with lower PHM expression. One non-peptide substrate analogue which is an irreversible covalent inhibitor of PHM appeared to be toxic to all cells when tested in the MTT assay. Further studies using this inhibitor in a clonogenic assay showed that the inhibition could be overcome by exogenous addition of the product of the inhibited enzymatic reaction (i.e., amidated GRP peptide hormone). We are therefore using the clonogenic method to test a range of non-peptide PHM inhibitors for maximal *in vitro* effect.

Proposed course

Studies on the biochemistry of PAM address the mechanism of regulation of activity of these enzymes in endocrine tissues, and will be extended to proliferating pre-neoplastic cells. Molecular biological studies on endoprotease processing enzymes are designed to develop probes for in situ hybridization studies of tumor tissue samples, and to yield protein sequences for antibody development. These reagents will be used for analysis of tumor tissue, and for early detection of pre-neoplastic tissues. The preliminary results obtained with the PHM inhibitors are being extended to determine which reagents would be best suited for therapeutic evaluation as cancer intervention and treatment agents.

Significance to biomedical research and DCPC

This project is designed to yield novel early detection and intervention tools, based upon an understanding of the biochemistry and biology of peptide hormone post-translational processing mechanisms. Early steps in the process of neoplastic transformation may include growth factor mediated proliferation. The enzymes involved with peptide hormone production therefore would provide novel targets for early detection of tumor cells. Furthermore, knowledge of the biochemistry of these enzymes should enable design and testing of compounds which may be useful as therapeutic and/or intervention agents for tumor cells.

BIOMARKERS AND PREVENTION RESEARCH BRANCH STAFF

Branch Chief	James L. Mulshine, M.D.
Deputy Branch Chief	Frank Cuttitta, Ph.D.
Section Chief	Terry Moody, Ph.D.
Section Chiefs (Proposed)	Michael Birrer, M.D., Ph.D. Ilona Linnoila, M.D.
Senior Investigators	Anthony M. Treston, Ph.D. Gail Shaw, M.D. Powel Brown, M.D., Ph.D. Sonia Jakowlew, Ph.D. Eva Szabo, M.D.
Research Associates	Anita Sabichi, M.D.
Senior Staff Fellow	John Jones, Ph.D.
Guest Researchers	Frank Scott, Ph.D. Kathyrn Quinn, Ph.D. Masahito Ebina, M.D., Ph.D.
Visiting Fellow	Noamichi Iwai, M.D.
Visiting Scientist	Jun Zhou, M.D. Sung Kim, M.D. Alfredo Martinez, Ph.D.. I. Gozes, M.D.
Cancer Prevention Fellow	Stephen Lemon, M.D.
Clinical Trials Specialist	Ruby Phelps, B.A.
Research Nurse	Cynthia Boland, R.N.
Chemist	Edward Unsworth, B.S.
Technicians	Ingalill Avis, R.N. Michelle Vos, B.S. Sandra Jensen, M.S. Mae Jean Miller, M.D. Lynda Nader, B.A. Theresa Chen Askale Mathias Charles Corbett
Secretaries	Ann E. Rogers, A.A. Regina Mease Ruth Miehm-Rowan

Summer Students	Michael Bachman Trisha Francis Julie Francis Daniel Gebhardt Hasan Zia Philip Chung Shuvo Ghosh
Stay-in-School	Doreen Francis

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Intramural Project Summaries

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00100-11 CPSB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Alpha-Tocopherol, Beta-Carotene Lung Cancer Prevention Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Albanes	Medical Officer	CPSB, DCPC, NCI
Others:	P. R. Taylor	Branch Chief	CPSB, DCPC, NCI
	B. K. Edwards	Associate Director	SP, DCPC, NCI
	A. M. Hartman	Health Statistician	ARB, SP, DCPC, NCI
	S. B. Green	Section Chief	CDTS, BB, DCPC, NCI

COOPERATING UNITS *(if any)*
National Public Health Institute, Helsinki, Finland
Surveillance Program, DCPC
Biometry Branch, DCPC

LAB/BRANCH
Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Alpha-Tocopherol, Beta-Carotene Lung Cancer Prevention Study (ATBC Study) is investigating the efficacy of daily oral alpha-tocopherol (50 mg) and beta-carotene (20 mg) in a double-blind, randomized 2x2 factorial design trial aimed at preventing lung cancer among 50-69 year old male cigarette smokers. The project is based on experimental and epidemiological research which demonstrates a potential preventive role for these agents. Recruitment took place between 1985-88, and the trial intervention ended on schedule March 31, 1993 after an average followup of over 6 years. A postal survey screening for potential trial participants was sent to 291,000 men in southern Finland, and 76% responded. We invited the smokers willing to participate (43,000) to one of 13 study clinics, and over 29,000 were randomized into the study. Compliance to the one capsule daily regimen has remained very high (97% average), and the dropout rate averages less than 6% per year. Reduction of lung cancer incidence in the active agent groups is the primary study goal; differences in the occurrence of other cancers will also be evaluated. Several pilot studies in support of the trial have also been completed including a feasibility study, validation of study dietary questionnaires, and evaluation of skin yellowing and serum levels following beta-carotene administration.

This trial is being conducted collaboratively with the Surveillance Program and the Biometry Branch of the Division of Cancer Prevention and Control and with the National Public Health Institute of Finland.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CN 00101-11 CPSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human Studies of Diet and Nutrition

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. R. Taylor	Branch Chief	CPSB, DCPC, NCI
Others:	A. Schatzkin	Medical Officer	CPSB, DCPC, NCI
	E. Lanza	Nutritionist	DCB, DCPC, NCI
	B. H. Patterson	Mathematical Statistician	BB, DCPC, NCI
	B. K. Edwards	Biostatistician	SP, DCPC, NCI
	M. Forman	Nutritional Epidemiologist	CPSB, DCPC, NCI
	W. Campbell	Research Study Coordinator	CPSB, DCPC, NCI
	M. Maher	Nurse Specialist	CPSB, DCPC, NCI
	C. C. Brown	Section Chief	BMCCES, BB, DCPC, NCI
	L. Yong	Staff Fellow	CPSB, DCPC, NCI
	B. I. Graubard	Mathematical Statistician	BB, DCPC, NCI
	J. Dorgan	Senior Staff Fellow	CPSB, DCPC, NCI

COOPERATING UNITS *(if any)*

U.S. Dept of Agriculture, Beltsville Human Nutrition Research Center
Surveillance Program, Biometry Branch, and Diet and Cancer Branch, DCPC
Armed Forces Institute of Pathology (M. Micozzi)

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	4.25	0.75

CHECK APPROPRIATE BOX(ES)

☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of dietary factors in cancer prevention has been assessed in animal experiments, in human epidemiologic studies, and most recently, in prevention trials. For many of these agents, however, information is incomplete concerning their safety, toxicity, dose, form, bioavailability, pharmacokinetics, and mechanisms of action. To further define these parameters in humans, a cooperative research effort between the Beltsville Human Nutrition Research Center (BHNRC), U.S. Department of Agriculture, and the CPSB, DCPC, is being conducted. Initial efforts have focused on three nutrients which have shown the most promise for cancer prevention: selenium, fat, and beta-carotene.

A study of the kinetics of a single, oral dose of two forms of selenium in the fasting and nonfasting state was conducted in the first year. Current activities include evaluations of the safety/toxicity of selenium and form of ingestion among persons residing in seleniferous areas.

Studies examining the metabolic effects of changes in dietary fat and fiber have been conducted separately in premenopausal women, postmenopausal women, and men. These dietary changes are being related primarily to serum lipids, hormonal status, bile acid metabolism, and fecal mutagenicity.

Beta-carotene studies are examining the plasma carotenoid response to single and long-term ingestion of beta-carotene from either a capsule or from selected vegetables.

Studies of the effects of alcohol on hormonal status and of the influence of omega-3 fatty acids on prostaglandins and the bioavailability of vitamin C have been completed.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00103-11 CPSB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Use of Isotretinoin in Prevention of Basal Cell Carcinoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. A. Tangrea	Deputy Branch Chief	CPSB, DCPC, NCI
Others:	P. R. Taylor	Branch Chief	CPSB, DCPC, NCI
	B. K. Edwards	Associate Director	SP, DCPC, NCI
	A. M. Hartman	Health Statistician	ARB, DCPC, NCI
	G. Peck	Senior Investigator	DB, DCT, NCI
	C. C. Brown	Section Chief	BMCCES, BB, DCPC, NCI

COOPERATING UNITS *(if any)*
Walter Reed Army Med Ctr; Fitzsimmons Army Med Ctr; Brooke Army Med Ctr; Eisenhower Army Med Ctr; Portsmouth Naval Med Ctr; Northwestern U; U of Arkansas; Roswell Park Med Inst; Dermatology Br, NCI; Radiology Dept, Clinical Ctr; Applied Research Branch, Surveillance Program, DCPC; Biometry Br, DCPC

LAB/BRANCH
Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study is a 5-year, randomized, double-blind clinical trial designed to evaluate the effectiveness of low dosage levels of isotretinoin in reducing the incidence of basal cell carcinoma in a high-risk population, and to examine possible side effects associated with long-term administration of low doses of isotretinoin. A total of 981 subjects were entered over 36 months at 8 participating clinical centers located around the country. At each center, subjects were randomly allocated to intervention (10 mg/day) or control (placebo) groups. Active intervention concluded in June 1990.

The rationale for this study includes the following. Laboratory experiments have shown that retinoids administered to animals can prevent chemical carcinogenesis. In the experimental animals, retinoids were effective even if administered after exposure to the carcinogen, and therefore the prophylactic effect of the retinoids is believed to be in the postinitiation phase, i.e., during the promotion phase of carcinogenesis. Recent case reports have shown that isotretinoin can prevent the appearance of new basal cell carcinoma for 4 years in patients at higher risk of developing new tumors.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00104-11 CPSB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
NHANES I Epidemiologic Followup Survey: Chemoprevention/Nutrition Aspects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. R. Taylor	Branch Chief	CPSB, DCPC, NCI
Others:	D. Albanes	Medical Officer	CPSB, DCPC, NCI
	A. Schatzkin	Medical Officer	CPSB, DCPC, NCI
	J. Dorgan	Senior Staff Fellow	CPSB, DCPC, NCI
	L. Yong	Staff Fellow	CPSB, DCPC, NCI

COOPERATING UNITS *(if any)*
This research developed as a collaborative effort by NCHS and various institutes at NIH: Biometry Branch, DCPC, NCI; NIH; NIMH; NIAAA, NHLBI; NINDS; NIDDK; NIAID; National Center for Health Statistics

LAB/BRANCH
Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of the NHANES (National Health and Nutrition Examination Survey) epidemiologic followup survey was to conduct a longitudinal study of 14,407 adults originally surveyed in 1971-75 and to investigate subsequent health and mortality outcomes. Respondents were traced and re-examined. Information was obtained from hospital records, the National Death Index, and death certificates. Several cycles have now been performed. The initial NHANES followup survey was completed in 1984. A continued followup of the elderly (75 years of age or older) in this cohort was conducted in 1985-86, while the entire cohort was again followed in 1986-87. Further followup in 1992 is planned.

The purpose of this intramural project is to examine the relation of chemopreventive, nutritional, and constitutional factors to cancer in the very large, representative population which NHANES offers. It provides an opportunity to examine these factors and potentially confounding or modifying factors in a prospective fashion, and to examine the effectiveness of dietary agents which are currently of great interest for cancer prevention. The relation of baseline vitamin use, biochemical or nutritional measures, and subsequent health status will be examined.

This study is being conducted by several of the National Institutes of Health and the National Center for Health Statistics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00105-11 BB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Research in Cancer Screening and Statistical Methodology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. C. Prorok	Section Chief	SS, BB, DCPC, NCI
Others:	R. J. Connor	Mathematical Statistician	SS, BB, DCPC, NCI
	S. G. Baker	Mathematical Statistician	SS, BB, DCPC, NCI
	K. Kafadar	Cancer Prevention Fellow	BB, DCPC, NCI
	D. L. Weed	Branch Chief	POB,EDCOP,DCPC,NCI
	B. L. Wells	Cancer Prevention Fellow	BB, DCPC, NCI
	D. Friedman	Epidemiology & Biostatistics Fellow	REB, DCE, NCI

COOPERATING UNITS *(if any)*
Early Detection Branch, DCPC, NCI; Radiation Epidemiology Branch, DCE, NCI; Department of Public Health and Social Medicine, Erasmus University, Rotterdam, the Netherlands (G. van Oortmarssen, R. Boer)

LAB/BRANCH
Biometry Branch, OD, DCPC

SECTION
Screening Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.1	0.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews
Analysis of data originally obtained from Human Subjects/Human Tissue

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The focus of this project is development and refinement of statistical procedures for the design and analysis of cancer screening and related studies. Problems under investigation include derivation and comparison of data analysis methods, assessment of case-control studies for screening evaluation, development of models of cancer screening, approaches to the analysis of categorical data, and geographic analysis and smoothing of cancer mortality rates. To assess the case-control design for screening evaluation, the MISCAN microsimulation model is being used to provide population data with and without screening. Case-control studies are then done in the screened populations and the results compared with the true effect to assess bias in the case-control approach. Criteria were developed for comparability of the restricted case subgroups used in the Limited Analysis of a cancer screening trial. Data from diagnostic testing and screening can often be analyzed using techniques for missing categorical data. A matrix model for incomplete multinomial data, the composite linear model, was developed to provide a unified approach for maximum likelihood inference for such data. A new study design for assessing screening was examined in which controls receive a screen at the end of the screening period, and only cases diagnosed to that time are followed and analyzed. Methods have been developed for estimating the benefit of screening unaffected by lead time bias and the average lead time, by examining the differences in case survival measured both from time of entry and time of diagnosis between screened and control groups. Approaches were defined for data monitoring of cancer screening trials. Further, exploratory analysis of cancer mortality rates for cancers of the lung, prostate, and skin was undertaken. Linear smoothing of standardized prostate cancer mortality rates revealed interesting features that otherwise might have been obscured.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00106-11 BB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies in Cancer Screening

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. C. Prorok	Section Chief	SS, BB, DCPC, NCI
Others:	R. J. Connor	Mathematical Statistician	SS, BB, DCPC, NCI
	S. G. Baker	Mathematical Statistician	SS, BB, DCPC, NCI
	J. K. Gohagan	Branch Chief	EDB, DCPC, NCI
	L. Kessler	Branch Chief	ARB, DCPC, NCI
	A. Coleman	Cancer Prevention Fellow	ARB, DCPC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Biometry Branch, OD, DCPC

SECTION
Screening Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	0.8	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Data from several cancer screening studies are being collected and analyzed to gain a better understanding of the impact and consequences of such screening in various population settings, and to develop new techniques for data analysis. Section staff are involved in design, monitoring, and data analysis aspects of these studies.

Screening Section investigators collaborated with the Early Detection Branch and the Research Contracts Branch in developing the major components of the PLCO Trial. This is a major trial of cancer screening in males and females for four cancers that comprise more than 50% of the incidence and mortality of cancer: lung, prostate, colorectal, and ovarian cancers. The trial design calls for a total sample size of 74,000 males and 74,000 females between the ages of 60 and 74 who are to be divided at random into a screened group and a control group. The screening techniques to be used are annual digital rectal examination and prostate specific antigen for prostate cancer, annual chest film for lung cancer, three-yearly flexible sigmoidoscopy for colorectal cancer, and annual ovarian physical examination plus CA-125 marker and transvaginal ultrasound for ovarian cancer. Contractors were chosen for the Coordinating Center, ten Screening Centers, and the Laboratory. NCI staff and the investigators at the Centers and Lab developed the trial protocol. Entry of study participants and initiation of the Pilot Phase of the Trial is planned for October 1993.

The database from the HIP breast cancer screening trial was used to address several scientific and modeling issues. Issues under investigation included the magnitude and duration of benefit, age-specific effectiveness, and application to model development. Data from the NCI sponsored lung cancer screening trials were analyzed. Investigations included assessment of incidence and mortality information. Monitoring continued of a trial to evaluate testing for blood in the stool for the early detection of colorectal cancer.

Section staff are involved in the International Workgroup on Information Systems in Breast Cancer Detection. The goal is development of a database containing key data elements from countries doing breast screening that could be used jointly or individually by the countries for evaluation of breast cancer detection. An initial database questionnaire has been developed and definition of terminology has been refined. Initial collection of data was begun in several countries and several alternatives for data collection in the U.S. were considered. Evaluation of screening for neuroblastoma in infants was addressed in conjunction with investigators at the U. of Minnesota. Staff consulted in a controlled study to assess the measurement of urinary catecholamine metabolites VMA and HVA as screening tests for this tumor.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00107-11 BB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Design and Analysis of Pharmacokinetic Studies of Selenium

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. H. Patterson	Mathematical Statistician	BMCCES, BB, DCPC, NCI
Others:	L. A. Zech	Senior Scientist	LMMB, DCBD, NCI

COOPERATING UNITS *(if any)*

Laboratory of Mathematical Biology, DCBD
Cancer Prevention Studies Branch, DCPC

LAB/BRANCH
Biometry Branch, OD, DCPC

SECTION
Biostatistical Methodology and Cancer Control Epidemiology Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Selenium is a possible cancer preventive agent. A study is in progress to provide information on the pharmacokinetics of selenium in its prototype forms: sodium selenite and selenomethionine. This information is necessary for the determination of time and manner of administration.

Integrated kinetic models are being used to interpret the study data. Various body pools have been hypothesized and rates of exchange between them estimated, as well as residence times. The models indicate important kinetic differences between selenite and selenomethionine and they are being modified to be combined into a single model to better simulate dietary intake of selenium.

A workshop is being organized jointly with the Chemoprevention Investigational Drug Unit to review the current state of knowledge on the efficacy and toxicity of selenium compounds in preventing cancer in animals and humans; the workshop is being held in anticipation of the possible expanded use of selenium compounds as chemopreventive agents in clinical trials in humans. Leading selenium researchers have been invited to give presentations that will provide an overview of the current state of selenium research, with a focus on safety and efficacy, and to participate in roundtable discussions aimed at obtaining information necessary to submit NDAs (New Drug Applications) to the FDA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00112-10 CPSB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Nutrition Intervention Studies of Esophageal Cancer in Linxian, China

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PIs:	P. R. Taylor	Branch Chief	CPSB, DCPC, NCI
	W. Blot	Branch Chief	BB, DCE, NCI
Others:	J. A. Tangrea	Deputy Branch Chief	CPSB, DCPC, NCI
	S. Dawsey	Senior Staff Fellow	CPSB, DCPC, NCI
	S. Mark	Staff Fellow	BB, DCE, NCI
	M. Gail	Section Chief	BB, DCE, NCI

LAB/BRANCH
Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to conduct two intervention trials using multiple vitamin-mineral supplements to evaluate the relationship between such supplements and esophageal cancer incidence and mortality. One trial is being conducted in patients diagnosed with esophageal dysplasia (n=3,400) and the other in the general population in a high-risk region (n=30,000). The effect of these supplements on regression/progression of esophageal dysplasia and total cancer incidence, total cancer mortality, and total mortality will be evaluated. These two studies are being conducted in Linxian (Henan Province) in the People's Republic of China (PRC). Linxian, a rural country with population 800,000 was selected because it has the highest rate of esophageal cancer in the world (greater than 100/100,000) and because there is suspicion that the population's chronic deficiencies of multiple nutrients may be etiologically involved.

This study is being conducted jointly by the Biostatistics Branch of the Division of Cancer Etiology and the Cancer Prevention Studies Branch of the Division of Cancer Prevention and Control at the NCI in collaboration with the Cancer Institute of the Chinese Academy of Medical Sciences.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00113-10 BB

PERIOD COVERED
October 1, 1993 to November 26, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cancer in Oriental Populations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F. B. Locke	Health Statistician	BMCCES, BB, DCPC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Biometry Branch, OD, DCPC

SECTION
Biostatistical Methodology and Cancer Control Epidemiology Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of Oriental populations represent the Division's continuing interest in the health risk among these minority groups for the mapping out of cancer prevention and control programs:

1. An analysis correlating mortality from cardiovascular diseases in 65 mostly rural counties in mainland China with various diet and lifestyle measurements has been completed and accepted for publication.

2. Assembling mortality/incidence figures on Asian populations from various sources, we have established an international file of cancer/non-cancer causes since 1960. These Asian resource data, age-adjusted and age-specific rates, also include figures for U.S. and "homeland" populations.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CN 00116-10 BB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Statistical Methodology Research

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. B. Green	Section Chief	CDTS, BB, DCPC, NCI
Others:	D. K. Corle	Computer Systems Analyst	CDTS, BB, DCPC, NCI
	B. I. Graubard	Mathematical Statistician	CDTS, BB, DCPC, NCI
	P. Smith	Biostatistician (IPA)	CDTS, BB, DCPC, NCI
	M. G. Valsecchi	Guest Researcher	CDTS, BB, DCPC, NCI

COOPERATING UNITS *(if any)*
Applied Research Branch, SP, DCPC, NCI
National Center for Health Statistics
Information Management Services, Inc.

LAB/BRANCH
Biometry Branch, OD, DCPC

SECTION
Clinical and Diagnostic Trials Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.6	2.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to conduct research in statistical methods and computer techniques with particular emphasis on those appropriate for analyzing data from clinical, diagnostic, and prevention trials and epidemiologic studies of cancer. Many of the problems studied under this project arise from the consultative activities of the Section.

Important activities during the past year have included investigating methods for analyzing complex sample survey data, including ways of incorporating the clustering and weighting of the observations into regression analyses of epidemiologic studies, and approaches to analyzing data from large national health surveys such as the National Health and Nutrition Examination Surveys (NHANES) and the National Health Interview Surveys (NHIS); analyzing dietary survey data using a statistical model incorporating a mixture of abstainers and consumers, which relates abstention and average consumption to covariates; developing a computer model for assessing the effects of population-based cancer screening programs; using permutation tests for analyzing disease progression based on patient status measurements at fixed time points; and fitting proportional hazard models to survival data for patients with colorectal cancer to identify prognostic factors and thus to predict survival for future patients, as well as comparing this approach to other proposed statistical methods.

Finally, the Section has continued to maintain and improve software for interactive analysis of complex medical data using sophisticated multiple regression techniques and survival analysis. These programs are operational on the NIH Convex Computer system.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00119-10 BB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Consultation on Clinical Trials and Other Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. B. Green	Section Chief	CDTS, BB, DCPC,NCI
Others:	D. K. Corle	Computer Systems Analyst	CDTS, BB, DCPC,NCI
	B. I. Graubard	Mathematical Statistician	CDTS, BB, DCPC,NCI

COOPERATING UNITS *(if any)*
Early Detection and Community Oncology Program, Cancer Control Science Program, & Cancer Prevention Research Program, DCPC; Division of Cancer Treatment & Division of Cancer Etiology, NCI; Information Management Services, Inc.

LAB/BRANCH
Biometry Branch, OD, DCPC

SECTION
Clinical and Diagnostic Trials Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to provide consultation on statistical and epidemiological methodology in the design, interpretation, and evaluation of clinical trials of diagnosis, treatment, and prevention of cancer, and other studies requiring this kind of expertise. For some studies the Section provides full statistical support, including development of detailed study plans; assistance in the design of appropriate study forms; supervision of randomization (for trials); collection, processing, and editing of data; performance of interim analyses during the progress of the study; preparation of progress reports; final analysis of study data; and collaboration in the preparation of scientific papers.

During the past year the Section has continued to collaborate extensively on the Community Intervention Trial for Smoking Cessation (COMMIT). Key activities during this year were design and implementation of the final cohort and cross-sectional surveys, and planning the statistical analysis for major endpoints, involving the exploration of statistical techniques for adjusting observed differences using baseline prognostic factors and for handling missing data using baseline covariates and patterns of intermediate endpoints. The Section has continued to provide full statistical support for the randomized clinical trials of multimodality treatment conducted by the Brain Tumor Cooperative Group.

Other important activities this past year include consulting on design issues in large-scale randomized prevention trials relating to breast cancer and prostate cancer; analyses of data from feeding studies measuring intakes of carotenoids; and collaborating on a physician practice study, involving a three-arm randomized design, of the adoption and use of the NCI Primary Care Nutrition Guide.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00121-09 BB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Research in Biostatistical Methodology and Mathematical Modeling

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. C. Brown	Section Chief	BMCCES, BB, DCPC, NCI
Others:	V. Kipnis	Visiting Scientist	BMCCES, BB, DCPC, NCI
	L. S. Freedman	Acting Branch Chief	BB, DCPC, NCI
	A. Schatzkin	Medical Officer	CPSB, DCPC,NCI
	S. Wacholder	Mathematical Statistician	BB, DCE, NCI
	A. M. Hartman	Health Statistician	ARB, DCPC, NCI

COOPERATING UNITS *(if any)*
Cancer Prevention Studies Branch, CPRP, DCPC, NCI
Biostatistics Branch, EBP, DCE, NCI
Applied Research Branch, SP, DCPC, NCI

LAB/BRANCH
Biometry Branch, OD, DCPC

SECTION
Biostatistical Methodology and Cancer Control Epidemiology Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	0.9	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Different statistical methods to adjust the effect of macronutrient intake for total energy intake are currently being used to analyze epidemiologic studies of diet and disease. This research examines the statistical properties of these methods. Accepted for publication was a paper which interprets the regression coefficients of three alternative regression models; we show that four different effects of interest (related to either adding calories to the diet or substituting sources of calories in the diet) are estimable by each model and we derive the standard errors of these estimates. Our research also examines the behavior of these methods when the study subjects are categorized into a small number of groups according to their nutrient intake. When the true macronutrient intakes and their inter-relationships are known without error, one result of this investigation shows Willett's "residual" method to be more powerful than the "standard" method (both measuring the effect of calorie substitution) and very similar to the "density" method.

Many regression procedures involve multi-step model building based on the use of repeated tests of significance. Theory has been developed to address problems resulting from repeated testing for a forward selection procedure. After step 1, the commonly used F-ratio does not have a conventional F-distribution but an appropriate conditioning helps in developing the "correct" testing procedure.

Application of exploratory analyses for selecting the 'best' regression predictor model affects statistical properties of conventional Mean Square Error of Prediction estimators and, in particular, can lead to substantial bias. Different bootstrap-type estimators have been studied using theory and Monte-Carlo Simulations.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00142-09 BB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cancer Control Objectives and Cancer Mortality Projections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. L. Levin	Senior Research Investigator	BB, DCPC, NCI
Others:	L. G. Kessler	Branch Chief	ARB, SP, DCPC, NCI
	A. Potosky	Operations Research Analyst	ARB, SP, DCPC, NCI
	M. Brown	Economist	ARB, SP, DCPC, NCI
	E. Feuer	Mathematical Statistician	ARB, SP, DCPC, NCI
	P. Smith	Biostatistician	BB, DCPC, NCI

COOPERATING UNITS *(if any)*

Applied Research Branch, Surveillance Program, DCPC
Information Management Services, Inc.

LAB/BRANCH
Biometry Branch, OD, DCPC

SECTION
Office of the Chief

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Projecting cancer incidence and mortality rates, and relating those projections to the attainment of national cancer control objectives are the goals of this intramural research project. The project includes development and continued refinement of a computer model which projects cancer incidence and mortality, meshing together data from a variety of sources, and adapting quantitative cancer control objectives to fit the modeling framework.

The NCI staff has developed and written a large interactive Fortran program used to project cancer figures for a forty year period. The model incorporates different models for survival from cancer, includes data for a number of cancer sites, the ability to examine temporal trends in underlying cancer incidence and mortality from other causes, adjustment of rates to different populations, and production of annual projections of cancer incidence and mortality. The crux of the model is the flexibility to analyze the effect of cancer prevention, screening, and treatment activities (in any combination) on cancer mortality.

Work in the current year has involved continued updating of the basic underlying database used by the program, modifications related to implementation on the NIH Convex computer, calculation of rates to different standard populations, and coordination with a similar program developed for the IBM personal computer. One of the major efforts related to the IBM model involved modeling of cancer screening.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00143-09 CPSB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Continued Followup of the Breast Cancer Detection and Demonstration Project

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PIs:	A. Schatzkin	Medical Officer	CPSB, DCPC, NCI
	C. Schairer	Health Statistician	EEB, DCE, NCI
Others:	R. N. Hoover	Branch Chief	EEB, DCE, NCI
	L. A. Brinton	Section Chief	EEB, DCE, NCI
	L. Yang	Staff Fellow	CPSB, DCPC, NCI

COOPERATING UNITS *(if any)*
Environmental Epidemiology Branch, DCE
Early Detection Branch, DCPC (C. Smart)
Biometry Branch, DCPC (D. Corle)

LAB/BRANCH
Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Breast Cancer Detection and Demonstration Project (BCDDP) screening program began in 1973 in 29 centers in 27 widely dispersed geographic areas of the United States. Initial screening was complete on over 280,000 women over a 2-year period. From the original 280,000 participants in the screening phase of the BCDDP, approximately 64,000 were selected for 4 years of long-term followup (LTF) beginning in 1978, to assess the biology and natural history of breast disease, and to test hypotheses relating to detection, etiology, and survival. Those selected for LTF included all breast cancer cases found during the screening phase, all benign breast cancer cases, all those recommended for biopsy, and a sample of "normals." The LTF database will facilitate the exploration of important questions regarding the etiology and natural history of breast cancer. The size of the subcohorts and breadth of data available on them make this population unique. The large number of cases of both breast cancer and benign breast disease with histologic information available should allow particularly useful analyses of several risk factors in relation to these conditions.

The first 5 years of LTF was completed in all centers in September 1986, and further continued followup is in process.

This project is being conducted jointly by the Cancer Prevention Studies Branch of the Division of Cancer Prevention and Control and the Environmental Epidemiology Branch of the Division of Cancer Etiology.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00146-05 CPSB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Nutritional Factors and Cancer in the Framingham Heart Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Schatzkin	Medical Officer	CPSB, DCPC, NCI
Others:	J. Dorgan	Senior Staff Fellow	CPSB, DCPC, NCI

COOPERATING UNITS *(if any)*
Boston University and the National Heart, Lung and Blood Institute (NHLBI).

LAB/BRANCH
Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In recent years considerable interest has been focused on the possible relation between moderate consumption of alcoholic beverages and breast cancer in women. Five epidemiologic cohort studies and the majority of case-control studies have demonstrated a positive association between moderate alcohol consumption and breast cancer, with relative risks ranging from 1.5 to 2.0. Given the frequency of alcohol consumption among women in this country, even a risk elevation of 50-100% would translate into considerable breast cancer morbidity and mortality that would be attributable to drinking. Further epidemiologic investigation of this question is of high priority.

In this regard, the Division of Cancer Prevention and Control has funded a contract for the procurement of a cancer file based on the original cohort in the Framingham Heart Study. This ongoing prospective cohort study was initially set up to examine risk factors for coronary heart disease, stroke, and other cardiovascular endpoints. Data, including detailed information on alcohol consumption, have been collected for over 30 years. The creation of the cancer file has been successfully completed in the past year and is being used to examine a number of hypotheses relating nutritional factors to cancer, including alcohol use, body fat distribution, physical activity, and serum cholesterol.

A similar study (Z01 CN 00147-05 CPSB) is being conducted on children of the original cohort.

This study is being conducted collaboratively with investigators from Boston University and the NHLBI.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00147-05 CPSB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Nutritional Factors and Cancer in the Framingham Offspring Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Schatzkin	Medical Officer	CPSB, DCPC, NCI
Others:	J. Dorgan	Senior Staff Fellow	CPSB, DCPC, NCI

COOPERATING UNITS *(if any)*
Boston University and the National Heart, Lung and Blood Institute (NHLBI)

LAB/BRANCH
Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.50	0.25	0.25

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Framingham Offspring Study has been undertaken in order to explore the relation between alcohol and breast cancer. This cohort study consists of 5,135 children (2,646 female, 2,489 male) of the members of the original Framingham Heart Study Cohort. The baseline examination period was 1972-77 (Cycle 1). Subsequent followup periods were 1979-82 (Cycle 2) and 1984-5 (Cycle 3), with Cycle 4 currently ongoing. Alcohol consumption, both frequency and amount by type of beverage, has been ascertained at each cycle. Information on socioeconomic status, and reproductive and family history has been routinely collected. These additional data are important in controlling for variables that might confound an observed association between alcohol and breast cancer.

Six hundred cancers (300 in both men and women) are projected (based on the application of SEER rates to the cohort). This includes approximately 100 breast cancer cases in women, 110 lung cancers (80 in men), and 110 colorectal cancers (60 in men).

A similar study (Z01 CN 00146-05 CPSB) is being conducted on the original cohort.

This study is being conducted collaboratively with investigators from Boston University and the NHLBI.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00148-05 CPSB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Finland Studies of Nutrition and Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Albanes	Medical Officer	CPSB, DCPC, NCI
Others:	P. R. Taylor	Branch Chief	CPSB, DCPC, NCI
	B. K. Edwards	Associate Director	SP, DCPC, NCI
	A. M. Hartman	Health Statistician	ARB, DCPC, NCI

COOPERATING UNITS *(if any)*
National Public Health Institute, Finland
Social Insurance Institute, Finland
Applied Research Branch, Surveillance Program, DCPC

LAB/BRANCH
Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The important relationship of diet and nutrition in the development of cancer has become well known through various research efforts. Laboratory studies have shown cancer inhibitory function for various natural and synthetic nutrients in various models, which have been corroborated by human epidemiologic studies of nutrient intake, tissue levels, and cancer incidence. The objectives of these etiologic studies are to 1) assess the role of fats; selenium; and vitamins A, E, and C in breast cancer development; and 2) evaluate the relation of intake of various nutrients to subsequent cancer, particularly breast, colon, and lung. The project includes two studies. The first is a breast cancer case-control study of fats; total calories; selenium; and vitamins A, E, and C. The role of various anthropometric measurements, genetic markers for breast cancer, and reproductive factors are being explored. The second project is a comparison of nutrient intakes in cases and reference subjects identified from an existing large cohort with prediagnostic baseline dietary histories. Associations between various dietary components and several cancers will be assessed.

These studies are being conducted collaboratively with the Surveillance Program of the Division of Cancer Prevention and Control and the National Public Health Institute and Social Insurance Institute of Finland.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00149-05 CPSB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Yunnan Tin Miners Lung Cancer Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. R. Taylor	Branch Chief	CPSB, DCPC, NCI
Others:	A. Schatzkin	Medical Officer	CPSB, DCPC, NCI
	M. Forman	Nutritional Epidemiologist	CPSB, DCPC, NCI
	Y. L. Qiao	Visiting Associate	CPSB, DCPC, NCI
	B. I. Graubard	Mathematical Statistician	BB, DCPC, NCI
	M. M. Maher	Nurse Specialist	CPSB, DCPC, NCI

COOPERATING UNITS *(if any)*
Yunnan Tin Corporation
Cancer Institute, Chinese Academy of Medical Sciences
Division of Cancer Etiology, NCI
Biometry Branch, DCPC, NCI

LAB/BRANCH
Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As part of our general collaborative studies in China and the feasibility study for a lung cancer intervention study among Yunnan tin miners, two lung cancer case-control studies have been conducted among the tin miners. The first, a prevalence case-control study, interviewed 107 living cases diagnosed between 1967-84 and an equal number of matched controls. A second study includes 183 lung cancer cases incident in 1985 and 1986 among miners and an equal number of matched controls. Data concerning smoking, occupational exposures including radon and arsenic exposure, diet and other exposures were collected by personal interview. Analyses of risk by radon, tobacco, and arsenic in the prevalence study have been completed while analyses of the incident case-control study are ongoing.

These studies are being conducted collaboratively with scientists from the Cancer Institute of the Chinese Academy of Medical Sciences, the Labor Protection Institute of the Yunnan Tin Corporation, and the Division of Cancer Etiology at NCI.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00150-05 CPSB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Esophageal Cancer Genetics Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. R. Taylor	Branch Chief	CPSB, DCPC, NCI
Others:	S. Dawsey	Senior Staff Fellow	CPSB, DCPC, NCI

COOPERATING UNITS *(if any)*
Chinese Academy of Medical Sciences

LAB/BRANCH
Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The overall goal of this project is to develop an understanding of the genetic as well as environmental influences that are involved in the etiology of human esophageal cancer. In North Central China where rates of this cancer are highest in the world, a sample of families has been identified with extraordinary familial aggregation for the disease. The specific purpose of the first phase of these studies is to obtain existing pedigree and epidemiologic information on a limited number of these families, obtain additional data on the base population from which they were drawn, and initiate steps to prospectively follow these families for the development of cancer. Formal genetic and genetic/epidemiologic evaluations will include familial aggregation studies, studies of the transmission or segregation of the disease, and studies that compare lifestyle and dietary aspects between case and control families. Analyses of these data should provide a unique opportunity to understand the genetic and epidemiologic components of esophageal cancer.

This study is being conducted collaboratively by scientists at the Chinese Academy of Medical Sciences.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00151-05 CPSB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Dietary Intervention Study of the Recurrence of Large Bowel Adenomatous Polyps

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Schatzkin	Medical Officer	CPSB, DCPC, NCI
	E. Lanza	Nutritionist	CPSB, DCPC, NCI
Others:	L. S. Freedman	Acting Branch Chief	BB, DCPC, NCI
	C. Clifford	Health Scientist Administrator	DCB, DCPC, NCI
	L. Kruse	Research Study Coordinator	CPSB, DCPC, NCI
	J. Tangrea	Deputy Branch Chief	CPSB, DCPC, NCI
	R. Ballard-Barbash	Medical Officer	ARB, DCPC, NCI

COOPERATING UNITS *(if any)*
Biometry Branch and Diet & Cancer Branch, DCPC; U of Pittsburg (Pittsburg, PA); Kaiser Found Res Inst (Oakland, CA); Memorial Sloan Kettering Cancer Ctr (New York, NY); U of Illinois (Chicago, IL); Kaiser Found (Portland, OR); U of New York (Buffalo, NY); Walter Reed Army Med Ctr (Washington, DC); U of Utah (Salt Lake City, UT); and Edward Hines Jr. VA Hospital (Chicago, IL)

LAB/BRANCH
Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Large bowel adenomatous polyps present a unique opportunity to conduct an intervention trial because of the high prevalence rate in the general population, the high polyp recurrence rate in those who have undergone polypectomy, and the link between polyps and cancer. It is generally accepted that large bowel adenomas are a requisite precursor lesion for most large bowel cancers. Given the strong evidence for the polyp-cancer sequence, an intervention that reduces the recurrence of large bowel polyps would have a strong likelihood of reducing the incidence of large bowel cancer.

The major objective of this study is to determine whether a low fat, high fiber, high fruit and vegetable dietary pattern will decrease the recurrence rate of large bowel adenomatous polyps. This is a multi-center randomized controlled trial involving 2,000 men and women. Study participants are being randomized into either the experimental diet group or a control group (usual diet). Recruitment will take up to two years, and the followup time from randomization is four years.

The study has three secondary objectives: 1) to investigate the relation between the dietary intervention and several putative intermediate endpoints in large bowel carcinogenesis, particularly markers of colonic epithelial cell proliferation; 2) to evaluate whether these intermediate endpoints correlate with subsequent neoplasia (adenoma formation); and 3) to determine the extent to which changes in the intermediate endpoints account for the observed effect.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00153-04 CPSB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of the Effects of a Fat-Modified Diet on Hormones During Adolescence

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Dorgan	Senior Staff Fellow	CPSB, DCPC, NCI
Others:	A. Schatzkin	Medical Officer	CPSB, DCPC, NCI
	P. R. Taylor	Branch Chief	CPSB, DCPC, NCI
	B. H. Patterson	Mathematical Statistician	BB, DCPC, NCI

COOPERATING UNITS *(if any)*
National Heart, Lung, & Blood Institute; Children's Hospital (New Orleans, LA); Johns Hopkins U (Baltimore, MD); Kaiser Center for Health Res (Portland, OR); Maryland Med Res Inst (Baltimore, MD); Med College of New Jersey (Newark, NJ); Northwestern U (Chicago, IL); U of Pittsburgh (Pittsburgh, PA); U of Iowa (Iowa City, IA)

LAB/BRANCH
Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.25	0.25

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study is ancillary to the Diet Intervention Study in Children (DISC), sponsored by the Division of Epidemiology and Clinical Applications, National Heart, Lung, and Blood Institute (NHLBI). DISC is a multicenter, randomized clinical trial designed to evaluate the feasibility, safety and efficacy of a fat modified diet during adolescence to lower LDL-cholesterol. The NCI sponsored ancillary study will evaluate the effect of this fat modified diet on sex hormones during adolescence. The effect of the diet on total concentrations of hormones and bioavailable fractions of hormones will be determined. The NCI sponsored ancillary study will also identify characteristics of adolescents, including age, Tanner stage, anthropometric measures, physical activity and dietary intake that affect sex hormone levels and bioavailability of sex hormones.

Dietary goals for the intervention group are to limit fat intake to 28% of calories and increase the ratio of polyunsaturated to saturated fats to approximately 1. Cholesterol intake will be restricted to 75mg/1000 calories. Children in the control group follow their usual diets.

This study is being conducted collaboratively with scientists from the National Heart, Lung, and Blood Institute in Bethesda, MD; Children's Hospital in New Orleans, LA; the Johns Hopkins University in Baltimore, MD; the Kaiser Center for Health Research in Portland, OR; the Maryland Medical Research Institute in Baltimore, MD; the Medical College of New Jersey in Newark, NJ; Northwestern University in Chicago, IL; the University of Pittsburgh in Pittsburgh, PA; and the University of Iowa in Iowa City, IA.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CN 00154-04 CPSB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Fels Early Nutrition and Growth Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: D. Albanes Medical Officer CPSB, DCPC, NCI

COOPERATING UNITS *(if any)*
Wright State School of Medicine

LAB/BRANCH
Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is designed to investigate the relation of childhood nutrition to breast cancer risk factors, including age at menarche, adult height, weight, and fatness. Secondary purposes include tracking the development of overweight and obesity from birth through young adulthood, identification of possible "sensitive" or high-risk periods (with respect to obesity) in childhood and, more important, to identify the contribution of diet to the development of childhood and adult obesity.

Detailed anthropometric data (height, weight, skinfold thickness, etc.) and demographic characteristics available from a computer data base from the Fels Study and the Division of Human Biology of the Wright State School of Medicine included up to 18 annual dietary and anthropometric assessments for 106 girls. Calorie, macro- and micronutrient data were linked to the existing anthropometry computer file, including later adult height and weight. Nutrient composition has been calculated using the latest version of the USDA Handbook series. Nutrients include the following: total energy (kilocalories); total fat, protein, and carbohydrate; saturated, polyunsaturated, and monounsaturated fat; cholesterol; dietary fiber; and vitamins and minerals (from food and supplementary sources).

This study is being conducted collaboratively with scientists at the Wright State School of Medicine in Yellow Springs, Ohio. Data analysis is currently underway.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CN 00155-03 LNMR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A New Mechanism for Carcinogen Resistance: Regulation by Diet and Nutrients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. C. Yeh	Senior Investigator	LNMR, CPRP, DCPC, NCI
Others:	J. M. Phang	Lab Chief	LNMR, CPRP, DCPC, NCI
	J. Lopaczynska	Visiting Fellow	LNMR, CPRP, DCPC, NCI
	J. Critchfield	IRTA Fellow	LNMR, CPRP, DCPC, NCI
	M. Poore	Biolab Technician	LNMR, CPRP, DCPC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.75	2.75	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our work has presented two lines of evidence suggesting that chemical carcinogen efflux mediated by the multidrug resistant (MDR) glycoprotein 170 (P-gp) may be an important mechanism in diet-dependent cancer prevention. First, we showed that benzo(a)pyrene and 7,12-dimethyl-benzanthracene efflux is mediated by P-gp. Efflux of these carcinogens was markedly higher in MDR cells than in their parent wild-type cells. Additionally, this difference was abrogated by MDR reversal agents, i.e., verapamil and quinine. Second, we showed in MCF-7 breast cancer cells and HCT-15 colon cancer cells that diet-derived flavonols markedly stimulated the P-gp mediated efflux of DMBA and adriamycin, respectively. Flavonols, e.g. galangin, queracetin and kaempferol, are widely distributed in fruits and vegetables and are known to inhibit tumorigenesis. Although P-gp, a product of the *mdr* gene, is known to play a critical role in cellular resistance to cytotoxic drugs, its function in normal cells has not been defined. We proposed that P-gp mediated efflux of carcinogens and the modulation of this efflux by dietary factors, e.g. flavonols, may be an important mechanism in cancer prevention.

While P-gp expression in cultured cells has been shown to be enhanced by retinoic acid and sodium butyrate, a functional role for diet-derived constituents in modulating the functional role for increased P-gp expression has not been demonstrated. We are currently examining the effects of flavonoids, retinoic acid and other dietary factors on P-gp expression. We propose that P-gp mediated carcinogen efflux is regulated by flavonoids as well as other dietary factors. These nutritional and molecular regulation of P-gp mediated efflux mechanisms are under current investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00156-03 LNMR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Nutritional Regulation of Carcinogens in Placenta-Related Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. A. Plouzek	Senior Staff Fellow	LNMR, CPRP, DCPC, NCI
Others:	G. C. Yeh	Senior Investigator	LNMR, CPRP, DCPC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The plasma membrane glycoprotein 170 (P-gp) responsible for multidrug resistance, MDR, also may function as an efflux pump for chemical carcinogens and can be regulated by diet and nutrients. P-gp is found predominantly in the cells lining the luminal space of a variety of normal tissues, including the placenta and the endometrium of the gravid uterus. The functional role of P-gp in normal tissues has not been determined. However, our recent findings indicate that P-gp mRNA is developmentally regulated in human placental tissues. Currently, a developmental study in rats of P-gp expression in normal tissues during gestation is in progress. Rat placentas, ovaries, uteri, adrenals and kidneys at 0, 6, 9, 12, 15 and 18 days of gestation are being processed for P-gp expression at the mRNA and protein levels. In addition, a SV40-temperature sensitive *A* (*tsA*) rat placental cell line is used to examine the effects of nutrients and diet factors in P-gp regulation.

A doxorubicin-resistant rat placental cell line has been established as a model for examining P-gp function in the defense against carcinogens. We will study the regulation of P-gp by nutrients and carcinogens in wild-type and drug-resistant placental cells at the protein and mRNA levels. In addition, we are studying the regulation of P-gp in a human endometrial adenocarcinoma cell line and a human cervical carcinoma cell line. Dietary effectors such as retinoic acid, known to enhance P-gp expression, and the flavonoid, kaempferol, are being investigated in the human endometrial and cervical cell lines. Special attention is paid to the two forms of MDR genes (*mdr* 1 and *mdr* 3) and their differential regulation by dietary factors and carcinogens in order to understand the physiologic regulation of P-gp.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CN 00157-03 LNMR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Effect of Proteins, Peptides, and Amino Acids on Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. M. Phang	Lab Chief	LNMR, CPRP, DCPC, NCI
Others:	G. C. Yeh	Senior Investigator	LNMR, CPRP, DCPC, NCI
	J. P. Henry	IRTA Fellow	LNMR, CPRP, DCPC, NCI
	S. J. Downing	Chemist	LNMR, CPRP, DCPC, NCI

COOPERATING UNITS *(if any)*
Johns Hopkins School of Medicine, Baltimore, MD (D. Valle)

LAB/BRANCH
Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.4	1.4	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Modulation of cellular signaling mechanisms by qualitative differences in dietary proteins and their metabolites were studied at the level of:

a) Imidodipeptides,
b) Pyrroline 5-carboxylate reductase as a mediator of redox exchange, and
c) Effect of pyrroline 5-carboxylate on mitogenesis-effect on membrane phosphoinositides

a. Imidodipeptides. Dipeptides containing proline or hydroxyproline originate from either tissue matrix degradation or from protein nutrition. They circulate in plasma and are delivered to tissues where they are hydrolyzed by prolidase. Thus, prolidase is a potential interface between protein nutrition and matrix breakdown. Our studies showed that the level of cellular prolidase is regulated by extracellular collagen acting through integrin receptors. Thus, the hydrolysis of imidodipeptides, the final degradative products of matrix collagen, is responsive to cellular interaction with extracellular matrix. We are studying the regulation of this enzyme on the molecular level.

b. Pyrroline 5-carboxylate reductase. We are studying this enzyme, which catalyzes the committed step in proline biosynthesis, on the molecular level. Previous studies suggested that it also functions in plasma membrane redox transfers. Using Western blots to analyze cellular fractions, we have shown that the enzyme is associated physically with cellular plasma membranes. The molecular mechanism for this association is being investigated.

c. Effect of pyrroline 5-carboxylate on mitogenesis. P5C stimulates PRPP and purine ribonucleotide synthesis synergistically with platelet-derived growth factor. It also increases the incorporation of thymidine in serum-activated cells. Inhibitor studies suggest that the effect is due to the turnover of membrane phosphoinositides. Direct assays show that it is phospholipase D which is stimulated by P5C with the release of phosphatidic acid.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00158-03 LNMR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Dietary Lipids and Signal Transduction in Breast Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. J. Welsh	Senior Staff Fellow	LNMR, CPRP, DCPC, NCI
Others:	J. M. Phang	Lab Chief	LNMR, CPRP, DCPC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This goal of this project is the elucidation of mechanisms relating dietary lipids as well as other nutrients and dietary factors to mechanisms of membrane signaling. Experiments using human breast cancer cells (MCF-7) to examine the role of membrane lipids in the generation of signaling lipid mediators have generated significant information in two areas. First, MCF-7 cells lack the ability to synthesize ether-linked phospholipids. The deficiency results from the failure of the alkyl dihydroxy acetone phosphate synthetase enzyme system to use fatty alcohol as a precursor for the ether-linked lipids. This cellular system represents a model to study the cellular physiology of ether-linked lipids. Second, multidrug resistant MCF-7 cells show an increase in phospholipase D activity (about 4x over drug sensitive controls) in response to phorbol ester. Pyrroline 5-carboxylate, a physiologic mediator of redox transfers, appears also to be a potent stimulator of phospholipase D. Gas chromatography/mass spectrometry is being used to characterize the phospholipase D response in the tumorigenic MCF-7 cells and in a "normal," nontumorigenic human breast cell line. These studies are expected to define the role that lipid mediators, such as phosphatidic acid and diacylglycerol, play in regulating mitogenic responses of breast cells. The impact of dietary fat, simulated by experimentally modifying the cellular membrane lipid composition, will be investigated with regard to the regulation of phospholipase-generated lipid mediators. Collectively, results from these experiments will elucidate the function of membrane lipids in cellular growth control. Future studies will emphasize the effects of trace fatty acids and fatty acid conjugates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00159-03 LNMR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Tumor Suppressor Protein p53

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Wang	Senior Staff Fellow	LNMR, CPRP, DCPC, NCI
Other:	J. M. Phang	Lab Chief	LNMR, CPRP, DCPC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously shown that mutation of phosphorylation sites Ser 315 and Ser 392 to alanine did not alter the ability of p53 to inhibit the growth of tumor cell line SW480. It was proposed that p53 may serve as a transcriptional enhancer. To obtain more insight into the molecular events, we utilized a transfection assay which monitors the expression of a reporter gene, chloramphenicol acetyl transferase (CAT), to study the effects of mutated phosphorylation sites on p53 function. We found that removal of phosphorylation sites did not affect the ability of p53 to stimulate production of the CAT enzyme. Thus, it appears that phosphorylation of Ser 315 or Ser 392 does not directly modulate the functional activity of p53. Others have reported that accumulation of p53 protein accompanies the cellular response to DNA damage. It is possible that phosphorylation regulated by external or internal stimuli may affect p53 function indirectly by stabilizing the protein and allowing for its accumulation. We are in the process of exploring the possible effects of phosphorylation on p53 stability using *in vitro* and *in vivo* methods. In addition, we are also in the process of examining the effects of dietary compounds which may modulate p53 accumulation.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CN 00160-03 LNMR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Nutritional Regulation of *Ras* Proto-Oncogene Activity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. N. Perkins	Senior Staff Fellow	LNMR, CPRP, DCPC, NCI
Others:	J. M. Phang	Lab Chief	LNMR, CPRP, DCPC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ras-mediated escape from normal regulation appears to be a frequent event in the multi-step genesis of cancer. A number of *in vitro* studies have demonstrated interactions between *ras* and other proto-oncogene products, especially *myc* and the tumor suppressor p53. A useful model for the study of these interactions is the commercially available p53 "knockout" mouse, a transgenic mouse in which null p53 germ line mutations prevent the expression of either one or both alleles for p53. Such p53-deficient animals develop normally but are prone to early tumorigenesis; indeed, 75% of homozygotes develop spontaneous neoplasms by 6 months of age. These mice can be used to study the effects of p53 gene dosage and various diets on carcinogenesis, or they can be used as a model of accelerated carcinogenesis that does not require exposure to chemicals that initiate and/or promote tumorigenesis. Moreover, fibroblasts cultured from embryos with the various genetic backgrounds afford a powerful *in vitro* system for addressing some of the same issues. We will use cDNA probes for *ras*, *myc* and p53 to assess the expression of these proto-oncogene mRNAs in various tissues from transgenic mice. Studies in progress with Dr. S. Hursting are investigating the effect of caloric restriction (a potent but poorly understood dietary regimen that dramatically inhibits tumor development in rodents); these studies will determine how this dietary manipulation combines with the gene dosage of p53 to affect proto-oncogene expression. Another area of interest is the dietary effect of the monoterpene, limonene, a major component of orange oil that has been shown to inhibit tumorigenesis in animal models. Part of the action of limonene may be through its inhibition of the farnesylation of *ras* proteins; this post-translational lipid modification is required for location of *ras* to the plasma membrane and hence *ras* activity. Using the sensitive semi-quantitative assay for *ras* we previously developed, we will be able to examine the effects of limonene on *ras* in p53-deficient mice *in vivo* and *in vitro*.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CN 00161-03 LNMR

PERIOD COVERED: October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Role of Fiber and Hormone-Like Compounds in Cancer Prevention

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. Sathyamoorthy	Senior Staff Fellow	LNMR, CPRP, DCPC, NCI
Other:	J. M. Phang	Lab Chief	LNMR, CPRP, DCPC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION: NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Former Title: "Role of Fiber and Phytohormones in Cancer Prevention"

Epidemiologic studies suggest a lowered risk of hormone-dependent cancers among vegetarians. Vegetable and fruits contain lignans and isoflavones which can be converted to biologically active hormone-like substances by intestinal flora. The interaction of these compounds with endogenous hormones has not been evaluated and may be an important mechanism in cancer prevention. We have established an assay system that identifies estrogenic factors in the diet. We used MCF-7 human breast cancer cells since it is known that the transcription of the pS2 gene is directly controlled by the action of estradiol in this estrogen receptor positive cell line. The expression of pS2 RNA was monitored by Northern blot analysis using a non-radioactive DIG-labeled probe. The effect of various phytoestrogens including enterolactone, enterodiol, equol, nordihydroguiaretic acid (NDGA), genistein, kaempferol, daidzein and quercetin on pS2 expression in MCF-7 cells were studied. From our results, it appeared that equol, genistein, daidzein and kaempferol are able to elicit a strong pS2 response; enterolactone evokes a milder response while quercetin and enterodiol are inactive. The effect of these different compounds on cell growth corroborated their estrogenic effects on pS2 expression.

The ability of enterolactone, quercetin, genistein, NDGA and equol to compete with estradiol for binding to the estrogen receptor was also evaluated. Genistein, equol, and NDGA competed with estadiol for binding to its receptor while enterolactone and quercetin did not.

We have devised a sensitive assay to assay extracts of fruits and vegetables as well as to test various diet-derived components for estrogenic activity. Furthermore, we are planning to study the effect of various compounds on other cell lines derived from tissue other than breast. In addition, we will emphasize the mechanism of action of lignans and flavonoids at the molecular level.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00162-03 LNMR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effects of Vitamin A Nutriture and Synthetic Retinoids on Retinol Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. C. Lewis	Senior Staff Fellow	LNMR, CPRP, DCPC, NCI
Others:	J. M. Phang	Lab Chief	LNMR, CPRP, DCPC, NCI
	L. A. Zech	Senior Scientist	LMMB, DCBDC, NCI

COOPERATING UNITS *(if any)*

Laboratory of Mathematical Biology

LAB/BRANCH
Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cancer chemopreventive and chemotherapeutic role of retinoids has been demonstrated in a variety of studies. However, the clinical usefulness of retinoid therapies is not likely to be fully realized until basic aspects of their metabolism are better understood. Thus, the general focus of work in our laboratory has been twofold: first, to investigate at the molecular, tissue and whole body level, the mechanisms involved in the normal physiological metabolism of vitamin A. Secondly, we examined the effects of chemopreventive retinoids on normal vitamin A metabolism. Several long-term studies of retinol kinetics were performed in animals fed N-[4-hydroxyphenyl] retinamide (4-HPR) or all-*trans*-retinoic acid. Following IV injection of a physiologically radiolabeled dose of retinol, retinol tracer and tracee kinetics were monitored in plasma and tissues for up to 41 days. Kinetic parameters were determined using the SAAM/CONSAM computer modeling programs to carry out graphical analysis of tracer concentration curves. Analysis of data from the 4-HPR study demonstrated major alterations of "native" vitamin A metabolism. Mean plasma retinol levels were reduced in the 4-HPR group to one-third of controls. The fraction of the plasma retinol catabolized per day was nearly twice as high in the 4-HPR treated group. The amount of time that retinol molecules spent in the plasma before being lost from the system was cut nearly in half in the 4-HPR treated group and the amount of vitamin A retinol ultimately utilized in these animals was 33% less than that used by the control group. Studies investigating the mechanisms by which 4-HPR alters retinol kinetics are presently underway in our laboratory. The results thus far would suggest that long-term administration of 4-HPR markedly perturbs normal retinol metabolism in rats. Whether 4-HPR similarly alters human retinol metabolism with untoward clinical consequences deserves careful evaluation. We are presently developing the appropriate methodology using stable isotope forms of vitamin A to carry out turnover studies in humans.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00163-02 LNMR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms for Deranged Androgen Responsiveness in Prostate Cancer Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. M. Phang	Lab Chief	LNMR, CPRP, DCPC, NCI
Others:	J. P. Henry	IRTA Fellow	LNMR, CPRP, DCPC, NCI
	X. Y. Sun	IRTA Fellow	LNMR, CPRP, DCPC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Androgens (testosterone and its metabolites) play a large role in the normal growth and function of the prostate. However, changes in androgen metabolism or responsiveness to androgens have been implicated in the formation of benign prostatic hypertrophy and prostate cancer. The causes of these changes are not well understood. Studies were undertaken to determine what if any differences in androgen metabolism occur between androgen dependent and androgen independent prostate cancer cells.

Whole cell studies showed that in androgen dependent cells, added testosterone is primarily glucuronidated. The cellular level of total testosterone, i.e. testosterone plus its glucuronide, remain constant during the entire incubation period. The kinetics, regulation, and physiologic effect of glucuronidation is being investigated. Androgen-independent cells, on the other hand, metabolize testosterone predominantly to androsterone. Unlike the dependent cell line, very little androgen remains within the independent cell at the end of the incubation period. Assays in a cell-free system show that androgen dependent cells have high activity of UDP-glucaronyl transferase whereas enzyme activity is undetectable in androgen independent cells.

We are characterizing the expression of UDP-glucuronyl transferase mRNA in these cells at the RNA and protein levels. Since this enzyme is sensitive to regulation by dietary factors, we are studying the mechanisms responsible for these differences in androgen metabolism to link androgen responsiveness in the prostate to dietary factors in cancer prevention.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00164-02 BPRB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Molecular Mechanisms of Oncogene Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. J. Birrer	Senior Investigator	BPRB, EDCOP, DCPC, NCI
Others:	P. H. Brown	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	E. Szabo	Clinical Associate	BPRB, EDCOP, DCPC, NCI
	A. Sabichi	Clinical Associate	BPRB, EDCOP, DCPC, NCI
	L. Nader	Biologist	BPRB, EDCOP, DCPC, NCI
	T. Chen	Biologist	BPRB, EDCOP, DCPC, NCI
	S. Kim	Fogarty Fellow	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS *(if any)*
Division of Cancer Etiology, NCI (N. Colburn)
University of Arizona (G. T. Bowden)
University of California at San Diego (M. Karin)
Johns Hopkins University (C. Dang)

LAB/BRANCH
Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

FORMER PROJECT TITLE: "Biologic Properties of Nuclear Oncogenes"

Recent developments in the application of molecular biology to epithelial cancers have led to the identification of specific genetic lesions resulting in either activation or inactivation of key target genes. These genes, called oncogenes, are involved in various aspects of cell growth regulation and as such play major roles in the early carcinogenic processes of "initiation" and "promotion." It is now critical to understand the precise mechanisms by which these genes function so molecular or pharmacologic agents can ultimately be derived to alter or repress their effects.

The purpose of this project is to elucidate the biochemical and molecular mechanisms by which oncogenes transform mammalian cells. To this end, we have performed structure/function analysis on members of the *myc, jun* and *fos* oncogene families. These studies have revealed various structural aspects of these proteins which are necessary and sufficient for transformation.

Our studies of the *c-jun* oncogene revealed that in addition to the DNA binding and dimerization domains, the N-terminal transactivation domain is required for cellular transformation. In addition, the ability of *c-jun* to transactivate correlates with its ability to transform cells. Thus, *c-jun* appears to transform cells by regulating gene expression. Further, detailed mutation analysis of *c-jun* has demonstrated that phosphorylation of cJun at serines 63/73 results in increased transactivation and ultimately transformation. The phosphorylation of these sites occurs in part through a *ras/raf* dependent pathway which provides an important biochemical link between these oncogenes. More recent studies are aimed at a more detailed analysis on other c-*jun* post-translational modifications and their biochemical and biologic effects and parallel studies with the *c-fos* oncogene examining the relationship between phosphorylation and biologic activity.

Our studies of the *myc* oncogene have focused on comparing the transactivating and transforming activities of the *c-myc* and *L-myc* genes. By exon shuffling, we have demonstrated that *L-myc* transactivates and transforms much less efficiently than *c-myc* and this difference is localized to the second exon. More recent work has focused on the precise structural differences between these genes and their role in apotosis.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CN 00165-02 BPRB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Use of Transcriptional Factors as Targets and Agents for Chemoprevention

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. J. Birrer	Senior Investigator	BPRB, EDCOP, DCPC, NCI
Others:	P. H. Brown	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	E. Szabo	Clinical Associate	BPRB, EDCOP, DCPC, NCI
	A. Sabichi	Clinical Associate	BPRB, EDCOP, DCPC, NCI
	L. Nader	Biologist	BPRB, EDCOP, DCPC, NCI
	T. Chen	Biologist	BPRB, EDCOP, DCPC, NCI
	S. Kim	Fogarty Fellow	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS *(if any)*
University of Arizona (G. T. Bowden)
Johns Hopkins University (C. Dang)
University of California at San Diego (M. Karin)
Division of Cancer Etiology, NCI (N. Colburn and U. Rapp)

LAB/BRANCH
Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

FORMER PROJECT TITLE: "Dominant Negative Mutants Of *c-jun* and Their Biologic Activities"

Transcriptional factors are critical regulators of gene expression. It is clear that these factors control the expression of many genes and as such mediate the biologic effects of agents such as "tumor promoters."

The purpose of this project is to design mutants of transcription factors specifically aimed at inhibiting their biochemical and, most importantly their biologic functions. The AP-1 complex has been specifically implicated in mediating the biologic effects of the tumor promoters "phorbol esters." A major component of this complex is the *c-jun* oncogene. We have created a panel of dominant-negative mutants of *c-jun* which are able to inhibit the biochemical functions of this oncogene. These mutants include:

1) a transactivation mutant with a deletion of amino acids 2-122,
2) three DNA binding mutants including one with a point mutation at position 265, a deletion at positions 269-272, and one with an insertion of 3 amino acids at position 265,
3) a dimerization dependent mutant missing the Leucine zipper,
4) a transactivation mutant (deletion of amino acids 2-122) with a homodimerization domain only, and
5) a transactivation mutant with a heterodimerization domain only.

We have recently begun to test the ability of these mutants to inhibit biologic functions. A transactivation mutant has been shown to inhibit *Jun* and *Fos* oncogene transformation in addition to the *in vitro* transforming effects of the tumor promoter TPA. Further work with this mutant has demonstrated that it can inhibit a wide range of oncogene transformation, the effects of phorbol ester in *in vivo* model systems of "tumor promotion," and tumorgenicity of some mouse epidermal tumor cell lines.

Future efforts are aimed at further refining the potency and specificity of these mutants by creating smaller mutants with higher affinities for dimerization and DNA binding, and testing them in specific human tumor systems such as breast and lung cancers. In addition, we are designing delivery mechanisms which might make these agents more clinically applicable. Finally, we are expanding these studies to include other transcription factors such as CREB.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00166-02 BPRB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of TGF-β Isoforms in Human Lung Cancer Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. B. Jakowlew	Senior Investigator	BPRB, EDCOP, DCPC, NCI
Others:	T. W. Moody	Section Head	BPRB, EDCOP, DCPC, NCI
	A. Mathias	Technician	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS *(if any)*
George Washington University, Washington, DC (F. Zia)

LAB/BRANCH
Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION
Experimental Biochemistry Section

INSTITUTE AND LOCATION
National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	1.5	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Expression of transforming growth factor-betas (TGF-βs) 1, 2 and 3 was examined in cultured human lung cancer cells. Specific cDNA probes and antibodies for TGF-βs 1, 2 and 3 were used to study expression of these different TGF-β isoforms in both non-small cell lung cancer (NSCLC) cells and small cell lung cancer (SCLC) cells. Expression of TGF-β1 mRNA was detected in both cell types using Northern blot hybridization, with expression being generally higher in NSCLC cells. Furthermore, expression of TGF-β2 and 3 mRNAs was also detected in both NSCLC and SCLC cells, but at levels that were significantly lower than that of TGF-β1 mRNA. Expression of TGF-β type II and type III receptor mRNAs was also detected in both NSCLC and SCLC cells, with expression of both mRNAs being higher in NSCLC cells than in SCLC cells. The level of expression of TGF-β type II receptor mRNA was significantly higher than that of TGF-β type III receptor mRNA. Immunohistochemical staining of cultured NSCLC cells with TGF-β antibodies showed immunoreactive TGF-βs 1, 2 and 3 proteins. Our results demonstrate coordinate expression of the TGF-β isoforms and their receptors in human NSCLC cells, with expression of TGF-β1 mRNA and protein more prominent than that of TGF-βs 2 and 3 and TGF-β type II receptor mRNA more prominent than that of TGF-β type III receptor.

Expression of retinoic acid receptor (RAR) and retinoid X receptor (RXR) mRNAs was also detected in both NSCLC and SCLC cells. The level of expression of RAR-α, RAR-β and RAR-γ mRNAs was approximately equal in most NSCLC cells, while expression of RAR-α mRNA was equal to or greater than that of RAR-β mRNA and significantly higher than that of RAR-γ mRNA in most SCLC cells. Expression of RXR-α, RXR-β and RXR-γ mRNAs was approximately equal in both NSCLC and SCLC cells, with the relative abundance being RXR-γ greater than RXR-β greater than RXR-α. Retinoic acid increased expression of TGF-β1 and TGF-β2 mRNAs, but not TGF-β3 mRNA, in some NSCLC cells; retinoic acid had no effect on TGF-β mRNAs in SCLC cells. Immunohistochemical staining of NSCLC cells with antibodies specific for each TFG-β isoform showed increased staining for TGF-β1 and TGF-β2 in NSCLC cells after treatment with retinoic acid. Also retinoic acid significantly inhibited colony formation of several NSCLC cells.

The significance of the project is to increase the expression of one or more of the TGF-β isoforms in lung cancer cells by treatment with chemopreventive agents such as retinoic acid. Increased TGF-β production may be used to slow the proliferation of lung cancer cells.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00167-02 BPRB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular Differentiation in Normal and Neoplastic Respiratory Epithelium

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. I. Linnoila	Senior Investigator	BPRB, EDCOP, DCPC, NCI
Others:	J. E. Jones	Senior Staff Fellow	BPRB, EDCOP, DCPC, NCI
	S. M. Jensen	Biologist	BPRB, EDCOP, DCPC, NCI
	E. Unsworth	Chemist	BPRB, EDCOP, DCPC, NCI
	M. Ebina	Guest Researcher	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS *(if any)*
NCI-Navy Medical Oncology Branch, DCT, NCI (H. Oie and B.E. Johnson)
Surgery Branch, DCT, NCI (H. Pass and S. Steinberg)
University of California, Davis (H. Witschi)

LAB/BRANCH
Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our aim is to characterize the cellular differentiation associated with premalignant changes in respiratory epithelium. This has been studied at the level of:

A. Neuroendocrine differentiation. We have demonstrated that 15% of non-small cell lung carcinomas (NSCLC) express multiple neuroendocrine (NE) features. Our results indicate that these tumors are sensitive to chemotherapy. The role of NE differentiation in non-neoplastic epithelium is investigated.

B. Peripheral airway cell differentiation. We found 30% of the 400 NSCLC tumors examined to be positive for at least one of the peripheral airway cell (PAC) markers SP-A and CC10. They also formed a clinically distinct subset. Characterization of NSCLC cell lines expressing PAC markers is in progress. In order to define premalignant lesions, we are studying the response of PACs in non-neoplastic lung to pulmonary carcinogens, including tobacco specific nitrosamines.

C. Clara cell specific protein (CC10) also known as a PCB(a potent carcinogen)-binding protein. We have demonstrated that non-ciliated secretory cells, which are progenitor cells for the epithelium and NSCLC, express high levels of CC10, while only 10% of NSCLC are positive for CC10. Our preliminary results showed that in the presence of smoking related atypia the patterns of CC10 mRNA expression in non-neoplastic human lung were affected both in larger airways and alveoli, while changes in smaller airways were minimal. Changes involved both intensity and cellular distribution of mRNA. Further studies are in progress.

D. Oncogene expression. We have found overexpression of *c-myc* in a high number of NSCLC as well as in the progenitor cells in human lung by in situ hybridization. In a cohort of 120 NSCLC patients, overexpression of p53 tumor suppressor gene was correlated with shorter survival in a subset of patients. Molecular analysis of the potentially prognostic mutations, and the mutations in premalignant changes in the surrounding non-neoplastic lung is in progress.

The significance of the project is that the results will provide a rational basis for innovative approaches for early detection and intervention in human lung cancer.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00168-02 BPRB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
CYP1A1 Gene Regulation and Human Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. E. Jones	Senior Staff Fellow	BPRB, DCPC, NCI
Others:	R. I. Linnoila	Senior Investigator	BPRB, DCPC, NCI
	S. M. Jensen	Biologist	BPRB, DCPC, NCI
	E. Unsworth	Chemist	BPRB, DCPC, NCI

COOPERATING UNITS *(if any)*
NCI-Navy Medical Oncology Branch,DCT, NCI (H. Oie)
Surgery Branch, DCT, NCI (H. Pass)

LAB/BRANCH
Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.4	2.4	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cytochrome P450 isoenzyme *CYP1A1* is intimately involved in the metabolic activation of pulmonary procarcinogens and its expression is induced by components found in tobacco smoke condensate. The *CYP1A1* gene has been implicated as a risk factor in the etiology of lung cancer in heavy cigarette smokers. The role of human *CYP1A1* in lung carcinogenesis was analyzed at the level of:

A. General regulatory patterns in neoplastic and non-neoplastic lung. Oligonucleotide directed mutagenesis (ODM) of numerous potential transcriptional elements within the regulatory region of the human *CYP1A1* gene was carried out. Using a panel of well characterized human non-small cell lung cancer (NSCLC) cell lines, we have identified a unique pattern of expression of the *CYP1A1* gene that requires interaction with two copies of the transcriptional activator known as the aromatic hydrocarbon receptor at two widely separated DNA regulatory elements.

B. Activator-repressor interactions. Elucidation of the pattern of expression of the human *CYP1A1* gene involving two transcriptional activators has stimulated additional studies on the potential role of a transcriptional repressor or "silencer."

C. Feedback modulation. In certain mutant murine derived hepatoma cell lines, the *CYP1A1* gene product, aromatic hydrocarbon hydroxylase (AHH) was shown to play an autoregulatory role in *CYP1A1* gene expression. We are investigating the possibility that, in some NSCLC lines, the elevated basal levels of *CYP1A1* gene expression observed may be due to a similar mechanism.

D. Proto-oncogene interaction. Preliminary studies have identified a potential interaction between the regulatory region of the *CYP1A1* gene and the proto-oncogenes *c-fos* and *c-jun*.

The significance of the project is to elucidate the interactive role of genetically determined factors and chemical carcinogens in pulmonary carcinogenesis. The results will have diagnostic and prevention applications.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00169-02 BPRB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Rational Applications of Biomarkers in Clinical Trials

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. L. Shaw	Senior Investigator	BPRB, EDCOP, DCPC, NCI
Others:	M. J. Birrer	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	S. Jakowlew	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	M. Schiffman	Physician Epidemiologist	EES, EEB, EBP, DCE, NCI
	S. Lemon	Cancer Prevention Fellow	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS *(if any)*
National Naval Medical Center (B. Ghosh and W. Laskin)

LAB/BRANCH
Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A major challenge for the BPRB is to begin to apply specific biomarkers in a rational way to permit more effective early detection approaches. Our laboratory resources will permit an intensive characterization of biomarkers and the biologic effects of intervention agents in a pilot study setting. Multiple markers have been implicated in the pathogenesis of human malignancies. Point mutations in the *ras* oncogene have been identified in colorectal and lung carcinomas and have recently been identified in shed epithelial cells found in stool specimens from patients with colorectal carcinomas. Alterations in carbohydrate antigen expression have also been found in malignancies and may be useful markers of neoplastic change.

Shed epithelial cells in archived stool specimens from patients with documented colorectal cancer will be analyzed using polymerase chain reaction for oncogene mutations or activation, or changes in carbohydrate antigen expression. These findings will be correlated with the markers present in the archived surgical material. Blocks from patients entered on a case-control study of colorectal cancer conducted at the National Naval Medical Center, Bethesda, Maryland have been obtained and are being analyzed for the presence of *ras* mutations and p53 expression. They will also be examined for carbohydrate antigen expression. If preliminary results are promising, additional specimens from patients entered on this study at Walter Reed Army Hospital and George Washington University Hospital, Washington, DC will also be obtained. Assays on stool specimens from control subjects will also be performed to assess the usefulness of these markers to discriminate patients with colorectal cancer from controls without cancer. The information obtained will be coupled with the previously collected epidemiologic data and Tumor Registry data for survival information to determine the potential usefulness for screening or prognostic purposes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00170-02 BPRB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical Evaluation of New Intervention Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. L. Mulshine	Branch Chief	BPRB, EDCOP, DCPC, NCI
Others:	G. Shaw	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	C. Boland	Research Nurse	BPRB, EDCOP, DCPC, NCI
	R. Phelps	Clinical Trials Specialist	BPRB, EDCOP, DCPC, NCI
	F. Cuttitta	Deputy Branch Chief	BPRB, EDCOP, DCPC, NCI
	T. Moody	Section Head	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS *(if any)*
Biomeasure, Inc. (J. P. Moreau)
Nuclear Medicine Department, NIH Clinical Center (J. Carrasquillo)

LAB/BRANCH
Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We and others have demonstrated the role of gastrin releasing peptides (GRP) as an autocrine growth factor, and the weight of this evidence is consistent with GRP playing an important role in early cancer formation. We have evaluated the use of a neutralizing monoclonal antibody to block the effect of this growth factor in patients with advanced small cell lung cancer. This treatment is associated with no demonstrable toxicity, but only one patient had a significant anti-tumor response.

Based on this experience, we proposed to evaluate a new class of GRP antagonists that are synthetic peptides. This class of molecules may have better properties such as bioavailability and affinity than monoclonal antibodies. These molecules might also be tagged with a radioisotope to permit exact pharmacologic analysis. Synthetic peptide growth factor antagonists may be very useful for delivery as intervention agents, and we proposed to evaluate that possibility. This effort would be a model for the type of rational intervention agent research that the BPRB staff will conduct.

Several peptide antagonists have been identified by evaluating *in vitro* cytotoxicity with lung cancer cell lines. These same analogues have been shown to have consistent growth inhibitory effects *in vivo* with mouse xenografts. Prior to evaluation in humans, acute and chronic toxicology studies must be performed to evaluate for unexpected patterns of side effects. We have already devised an approach to optimal dose determination for anti-GRP monoclonal antibodies; we can extend this analysis to the peptide antagonists to determine the validity of this approach in a new system.

GRP is a molecule that serves as an excellent model of a neuropeptide effector that may be important as a mediator of tumor promotion dynamics in certain epithelium; as such it comprises an attractive target for intervention research.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00171-02 BPRB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biological Regulation of Lung Cancer Growth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. L. Mulshine	Branch Chief	BPRB, EDCOP, DCPC, NCI
Others:	F. Cuttitta	Deputy Branch Chief	BPRB, EDCOP, DCPC, NCI
	T. Treston	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	I. Avis	Biologist	BPRB, EDCOP, DCPC, NCI
	T. Moody	Section Head	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS *(if any)*
Walter Reed Research Institute (M. Jett)

LAB/BRANCH
Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.95	0.75	0.2

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have evaluated a number of compounds that influence the growth of lung cancer cells. We have reported previously on the autocrine role of gastrin release peptide, insulin-like growth factor, and transferrin--all of which stimulate growth for certain types of lung cancer. We have also shown that regulatory molecules such as glucagon and 13-*cis*-retinoic acid can inhibit the growth of a number of lung cancer cells lines. This experience has allowed us to focus on the signal transduction pathways most central to the process of cellular proliferation. In collaboration with Dr. M. Jett, we have recently presented data suggesting that 5-HETE, a product of 5-lipoxygenase activation may be a key intermediary in growth factor mediated growth stimulation of cancer cells. Since considerable information exists about the lipoxygenase pathway, we can potentially exploit the availability of existence of specific antagonists for application as biointervention tools. Systematic evaluation of the growth factor biology of early cancer cells may yield additional clues for the development of rational cancer intervention agents. Promising leads from *in vitro* models demonstrating significant anti-cancer effects with lung cancer cell lines will be followed up with evaluation of efficiency for *in vivo* model systems.

The most interesting *in vitro* leads will be evaluated for clinical application in Phase I and II studies conducted by the BPRB. An important part of that effort would be the identification of markers for intermediate end points analysis; these would accelerate the process of determining the benefit of this class of intervention tools.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00172-02 BPRB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunocytomorphic Test for Early Lung Cancer Detection CRADA# 35516

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. L. Mulshine	Branch Chief	BPRB, EDCOP, DCPC, NCI
Others:	F. Scott	Guest Researcher	BPRB, EDCOP, DCPC, NCI
	I. Avis	Biologist	BPRB, EDCOP, DCPC, NCI
	M. S. Tockman	Associate Professor	Johns Hopkins
	P. Gupta	Professor	University of Pennsylvania
	J. Tomita	Group Leader	Abbott Laboratories

COOPERATING UNITS *(if any)*
Johns Hopkins University, Abbott Laboratories, University of Pennsylvania, Illinois Cancer Council, Memorial Sloan Kettering Institute, University of Toronto, University of South Florida, University of Colorado, University of Texas at San Antonio, M.D. Anderson, Quebec Cancer Centre

LAB/BRANCH
Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.25	0.75

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have established a CRADA to prospectively validate the diagnostic accuracy of a lung cancer early detection approach. A clinical team of investigators from 11 institutions throughout the United States and Canada is accruing stage I resected lung cancer patients to a protocol where annual induced sputums will be acquired and immunostained. Ongoing patient followup will permit the eventual correlation of immunostaining status with clinical outcome (correlation of positive immunostaining with the development of lung cancer and vice versa). Immunostaining for this study will be done at the University of Pennsylvania, and data acquisition and analysis will be handled at Johns Hopkins University. As part of this effort, selected patients will undergo bronchoscopy, and their bronchial lavage fluids will be studied for the quantity and quality of growth factor expression. We have developed a variety of methods for assessing the proliferative capacity of bronchial lavage products in an effort to complement the sputum immunocytology approach in determining who is and is not at risk for manifesting lung cancer.

An archive of sputum and other clinical specimens remaining after the primary analysis will be conserved to permit rapid analysis of other new promising early detection markers. All CRADA funds are being expended to support the clinical trial, and none of these funds are being spent at the NCI.

Core analysis includes quantitation of autocrine growth factors such as GRP as well as more global assessment analysis of neuroendocrine activation by monitoring levels of peptidyl amidating monooxygenase (PAM) activity. This application builds upon the biology elucidated in our lab, establishing the role of this enzyme system in contributing to the chronic growth stimulation of neoplastic pulmonary epithelium. This work has major relevance in developing new early lung cancer detection approaches.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CN 00173-02 BPRB

PERIOD COVERED: October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Identification of Peptide Growth Factors That Regulate Tumor Proliferation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F. Cuttitta	Deputy Branch Chief	BPRB, EDCOP, DCPC, NCI
Others:	K. Quinn	Post-Doctoral Fellow	BPRB, EDCOP, DCPC, NCI
	E. Unsworth	Chemist	BPRB, EDCOP, DCPC, NCI
	M. Miller	Biologist	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS *(if any)*

University of Pittsburgh, Pittsburgh, PA (J. Siegfried)

LAB/BRANCH: Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION:

INSTITUTE AND LOCATION: National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.4	1.9	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

α-amidation is a post-translational modification that occurs in some peptide hormones and involves the substitution of -NH2 (amide) for -OH (free-acid) residues at the carboxy-terminal amino acid. This chemical alteration is enzymatically mediated, and distinct amino acid sequence motifs code for the post-translation event to occur. Since α-amidation tracks with biological activity, we have used amidation signal motifs to identify cryptic peptides found within the precursor proteins of established human growth factors. Applying this investigative strategy to proinsulin-like growth factor-IB, a potential peptide amide (IBE1) has been found in the E domain of the precursor. A synthetic peptide homolog of IBE1 was shown to induce trophic effects, in a dose related manner, on both normal and malignant pulmonary cells. The peptide's proliferative action was shown to be dependent on the integrity of the amidated carboxy-terminal amino acid, the synthetic free-acid derivative being an impotent mitogen. The IBE1 peptide, though derived from IGF-IB precursor, does not mediate its action through the type 1 receptor of IGF-I. In a receptor binding assay with *125*I-IBE1 neither insulin, IGF-I, IGF-II, nor GRP at 2μM concentration were able to block labeled ligand/receptor interaction. Scatchard analysis of IBE1 receptor expression on A549 cells (bronchioalveolar CA) gave a KD 2.8 x 10 to the -11 power and a receptor density of 1.5 x 10 to the 4th power per cell. Using polyclonal antisera to synthetic IBE1, we have demonstrated the existence of immunoreactive peptides with molecular weights of 25kDa, 12kDa, 8kDa, and 5kDa in small cell lung cancer cell lines. In addition, similar immunoreactive peptides have been identified in normal liver extracts from a variety of mammalian species, implicating evolutionary conservation. We are now following similar research strategies to identify alternative peptide amides which mediate tumor proliferation; these may represent rational biologic targets for the early diagnosis and prevention of malignant disease.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00174-02 BPRB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Identification of Peptide Growth Factor Binding Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F. Cuttitta	Deputy Branch Chief	BPRB, EDCOP, DCPC, NCI
Others:	E. Unsworth	Chemist	BPRB, EDCOP, DCPC, NCI
	M. Miller	Biologist	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent evidence has demonstrated that a variety of human peptide growth factors have associated with their function naturally occurring binding proteins (BP) which modulate the ligand mediated proliferative response. The best example of this interaction is seen with insulin-like growth factor I (IGF-I) and at least five distinct BPs (encoded on different genes) that regulate IGF-I/receptor binding. It has been previously shown that different IGF-I BPs can have dichotomous effects on IGF-I induced growth. Some BPs bind to IGF-I, causing alterations in the ligand's structural conformation that results in enhancing type I receptor affinity and augmenting IGF-I's proliferative effect; these are superagonists. In contrast, other BPs interact with IGF-I to induce a steric interference of type I receptor binding and thereby block IGF-I effects; these are antagonists.

As an extension of our recent report on a newly discovered mitogenic peptide (IBE1 peptide amide) found within the E domain of IGF-IB prohormone, we have investigated whether BPs exist for this ligand. Towards this end, we examined if plasma or serum from normal and diseased individuals (lung cancer patients) contained IBE1 BPs. Initial screening studies utilized *125*I-IBE1 as a labeled tracer to determine the presence of BPs. Plasma or serum samples were incubated with the labeled ligand over different time courses (2 hr, 4 hr, 24 hr) and assessed for the presence of BPs by agarose electrophoresis. Alterations in the electrophoretic mobility (EM) of the free ligand over the test samples were interpreted as a positive indicator of BP expression. In normal individuals there were dramatic differences in the electrophoretic profile observed between homologous plasma/serum samples. Plasma universally showed no altered EM shifts over the free-ligand. However, the sera of all 6 normal donors demonstrated the existence of at least three distinct BPs having EMs of 1.4 cm, 2.6 cm and 4.2 cm from the origin. Differences in the binding patterns observed between plasma and serum were not due to the presence of a chelator since NaCitrate at 1x, 2x and 10x concentration used to generate the plasma did not alter serum binding results. These BPs may represent naturally occurring regulators of IBE1 function, a possibility which we are now actively investigating.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CN 00175-02 BPRB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Post-Translational Processing Mechanisms in Tumor Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. M. Treston	Visiting Scientist	BPRB, EDCOP, DCPC, NCI
Others:	N. Iwai	Visiting Associate	BPRB, EDCOP, DCPC, NCI
	M. Foo	Biologist	BPRB, EDCOP, DCPC. NCI
	A. Martinez	Visiting Scientist	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	2.25	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Amidated peptide hormones are an important class of tumor growth factors in endocrine lung tumor cells and are potentially important in the regulation of tumor progression. Our studies of tumor cell enzymes required for processing of precursor prehormones to active peptide hormones are comprised of three parts:

A: Biochemistry of peptidyl amidating enzyme complex
B: Molecular biology of endo- and exo-protease enzymes
C: Effect of inhibiting peptidyl amidating enzyme on cell growth

A: Both enzymes (PHM and PGL) required for conversion of glycine-extended prehormones to active amidated peptide hormones have been shown to be present in endocrine lung tumor cells. We have extended our studies on the different forms of these enzymes present in endocrine lung tumor cell lines to both non-lung and non-endocrine lung tumor cell lines. The presence of these enzymes in these cell types suggests that post-translational processing of growth-stimulatory hormones in tumor cells is much more common than previously anticipated. Furthermore, comparison of expression of markers of the endocrine and neuro-endocrine phenotype in a range of cell lines suggests disparate mechanisms of regulation of different aspects of the endocrine phenotype.

B: Our studies on the expression of prehormone endo- and exo-proteases at the level of mRNA expression have shown relatively infrequent or low levels of expression of two endoproteases reportedly involved with prehormone processing. We are developing pcr-based methods which will distinguish between the various members of the endoprotease family and enable detection at low levels.

C: We have shown that chemical inhibitors which chelate copper, an essential cofactor of PHM, inhibit growth of a lung tumor cell line growth-dependent on an amidated peptide hormone (GRP). An irreversible substrate analogue inhibitor of PHM appeared toxic to cells in the MTT assay, but further studies using a clonogenic assay showed that inhibition could be overcome by exogenous amidated GRP. Further inhibitors are being evaluated to develop growth inhibitors which are more selective, with the intention of eventual clinical testing of the best agents as cancer intervention agents.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00176-02 CPSB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biologic Specimen Bank for Early Lung Cancer Markers in Chinese Tin Miners

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. R. Taylor	Branch Chief	CPSB, DCPC, NCI
Others:	M. Forman	Nutritional Epidemiologist	CPSB, DCPC, NCI
	Y. L. Qiao	Visiting Associate	CPSB, DCPC, NCI
	M. M. Maher	Research Study Coordinator	CPSB, DCPC, NCI
	S. B. Green	Section Chief	CDTS, BB, DCPC, NCI

COOPERATING UNITS *(if any)*
Yunnan Tin Corporation
Johns Hopkins University School of Hygiene and Public Health
Division of Cancer Etiology, NCI
Biometry Branch, DCPC, NCI

LAB/BRANCH
Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.25	1.5	0.75

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lung cancer is the leading cause of death from malignant neoplasms in the United States. Reduction in the mortality from this lethal malignancy will require reduction in the prevalence of risk factors and/or improved diagnosis and therapy. While relative survival rates for localized disease are dramatically better than for nonlocalized disease, most patients are not diagnosed early enough for present therapies to be effective. Advances in our understanding of the biology of lung cancer in recent years indicate that research to identify early markers of lung cancer may hold great promise for the reduction of lung cancer mortality. Numerous potential candidates for the early detection of lung cancer in sputum exist.

The tin miners at the Yunnan Tin Corporation (YTC) in China have an extremely high rate of lung cancer. Among high risk miners, defined as 40+ years old with 10+ years of underground mining and/or smelting experience, lung cancer rates exceed 1% per year. These extraordinary lung cancer rates result from combined exposure to radon, arsenic, and tobacco smoking in the form of cigarettes and/or bamboo water pipe.

The primary objective of this study is to establish a biologic specimen bank and data bank that can be used for the validation and refinement of potential early markers of lung cancer. Biologic specimens to be collected include sputum, blood, urine, and toenails. A secondary objective includes the establishment of a cohort for the study of environmental (including dietary) and genetic risk factors for lung cancer.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00177-02 BB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Research in Statistical Methodology and Consultation for Cancer Prevention

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. S. Freedman	Acting Branch Chief	BB, DCPC, NCI
Others:	D. L. Levin	Senior Research Investigator	BB, DCPC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Biometry Branch, OD, DCPC

SECTION
Office of the Chief

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.8	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to conduct statistical research and provide consultation to the Division for clinical trials, laboratory experiments, and epidemiological studies relevant to cancer prevention and control.

Research problems under investigation include statistical methods for validating intermediate endpoints for cancer research, where the statistical criteria for validation are being elucidated and their application to biomarker research are being described; design of nutritional cohort studies including two-stage designs that allow construction of a followup cohort with a greater variation of nutrient intake; design of studies to validate dietary assessment instruments, allowing repeated assessments of two different instruments, one of which is unbiased; use of Bayesian methods for monitoring clinical trials; studies on a large observational database of HIV-infected subjects with evaluation of natural history of disease; the use of caloric adjustment models in the analysis of nutritional epidemiology studies, with emphasis firstly on the interpretation of three commonly used models when nutrient intakes are expressed as continuous variables and secondly on the interpretation of the same models when nutrient intakes are categorized as belonging to a certain quantile of the population distribution. In the latter case the results show that categorization leads to surprising differences with higher relative risks obtained from the "standard" model than from the "Willett" model.

Statistical consultation is provided to numerous studies including the NIH Women's Health Initiative Clinical Trial, the Polyp Prevention Trial, the AARP Nutritional Cohort Study, and several projects of the DCPC Chemoprevention Program. The consultation has involved extensive contributions to study design, reviewing proposals for interpreting the studies and, in the case of the Polyp Prevention Trial, continual advice on the day-to-day operations and on data monitoring.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00178-01 LNMR

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of Caloric Restriction in Cancer Prevention

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Phang, M.D.	Lab Chief	LNMR, CPRP, DCPC, NCI
Others:	S. Hursting, Ph.D.	Cancer Prev. Fellow	LNMR, CPRP, DCPC, NCI
	S. Perkins, Ph.D.	Senior Staff Fellow	LNMR, CPRP, DCPC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION
NCI-Frederick Cancer Research & Development Center, Frederick, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Elucidation of the cellular/molecular mechanism(s) of caloric restriction (CR), which inhibits the development of a variety of spontaneous and experimentally-induced tumors in rodents, may provide important clues for human cancer prevention. We are using a p53-knockout transgenic mice as an *in vivo* model to explore the mechanisms underlying the anti-tumor effects of CR.

The p53 gene is the most commonly identified mutated gene in human tumors. Recent evidence suggests that the wild-type p53 gene product protects cells against mutations by mediating an arrest of the cell-cycle in response to DNA damage, facilitating repair of damaged DNA and preventing fixation of mutagenic lesions that can lead to neoplasia. Mice with the p53 gene knocked-out by homologous recombination develop normally but have increased susceptibility to spontaneous tumor development, with approximately 100% tumor incidence by 6 months of age in untreated homozygous p53-deficient mice. We have shown that CR markedly decreased the incidence and increased the latency of spontaneous tumor development in these mice.

Cellular and molecular studies on tissues collected serially from wild-type, homozygous and heterozygous p53-deficient mice fed *ad libitum* or CR-treated, are currently in progress. We have also begun analyzing tissues for differences in p53, *ras*, *fos/jun* and *mdr* expression using Northern blot analysis. We are also conducting a 2-stage skin tumorigenesis experiment with wild-type and heterozygous p53-deficient mice to determine the stage in the carcinogenesis pathway in which CR is exerting its effects and to further explore the mechanisms underlying the anti-tumor effects. The effect of caloric restriction in the presence and absence of the p53 gene on the development of papillomas and the progression of papillomas to carcinomas will be evaluated.

Finally, we have established *in vitro* embryonic fibroblast cell lines isolated from wild-type, homozygous and heterozygous p53-knockout mice to facilitate the evaluation of potential intermediates of the tumor-inhibitory effects of CR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00179-01 BPRB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Transcription Factors in Breast Epithelial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. H. Brown	Senior Investigator	BPRB, EDCOP, DCPC, NCI
Others:	M. J. Birrer	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	T. Chen	Biologist	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Over the last year, we have begun to investigate the role of nuclear transcription factors in controlling proliferation and transformation of human breast epithelial cells. We have demonstrated that the Jun and Fos families of transcription factors are expressed in a variety of nontumorigenic and tumorigenic breast epithelial cell lines, and have shown that Jun and Fos RNA expression, and transcriptional activating activity are stimulated by a variety of growth factors, and also by TPA. In addition, an inhibitor of Jun and Fos transactivating activity which effectively suppresses transcriptional activation in rat fibroblasts also inhibits Jun and Fos activity in human breast epithelial cells. Studies are now ongoing to determine if this inhibitor is capable of inhibiting the proliferation or transformation of these human breast epithelial cells.

Additional ongoing studies include the characterization of other transcription factors in human breast epithelial cells. We are presently studying the members of the CREB family which regulate the cellular response to cyclic AMP, and the C/EBP family which are involved in regulating the cellular response to calcium. A detailed characterization of their expression and activity in human mammary cells will allow us to determine the relative role of each of these transcription factor families in controlling cellular proliferation and transformation. Once the activities of these transcription factors are well characterized, we will modulate their activity using inhibitors specific for each family of transcription factors. By interfering with transcription factor function, we may be able to block signal transduction pathways at a distal point where the signals from multiple growth factors converge. If these specific transcription factor inhibitors affect proliferation or transformation in human breast cells, such inhibitors might be promising chemopreventative agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00180-01 BPRB

PERIOD COVERED
June 1, 1993 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of Markers for the Early Detection of Breast Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. L. Shaw	Senior Investigator	BPRB, EDCOP, DCPC, NCI
Others:	P. Brown	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	J. L. Mulshine	Chief	BPRB, EDCOP, DCPC, NCI
	E. Szabo	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	S. Jakolew	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	I. Avis	Biologist	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS *(if any)*
GlycoTech, Rockville, MD (J. L. Magnani)

LAB/BRANCH
Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.75	1.25	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

While mammography provides a method for the early detection of breast cancer, there are still breast cancer patients who do not have mammographically detectable lesions. Furthermore, only 25% of women who develop breast cancer have a recognized risk factor. Evaluation of the ductal epithelium of the breast may reveal markers which could identify women who are at an increased risk for developing breast cancer and thereby would derive greater benefit from surveillance or would be appropriate for intervention studies. Breast ductal epithelium is shed into ductal fluid, and this fluid can be aspirated from the nipple in approximately 50-60% of women. Published studies by Petrakis and others have shown that the ability to yield fluid is associated with an increased risk of breast cancer compared to non-yielders, but proliferative cytology alone is not adequate to predict breast cancer occurrence.

Evaluation of biomarkers on the breast epithelium detectable in breast duct aspirate may provide a method of early detection of cellular changes. During Summer 1993 a protocol will be submitted for a feasibility trial to determine the acceptability of obtaining breast nipple aspirate fluid, to characterize the range of volumes obtained and to develop methods of performing multiple assays of the fluid obtained. Specimens will be examined for expression of markers including IGF-1 or TGF-β levels, estrogen and progesterone receptors, retinoic acid receptors, erbB-2 expression, carbohydrate antigen expression and others. Breast duct aspirate and breast needle aspirates would be obtained to characterize marker expression detectable in one or both specimen sources for concordance. An intervention trial with tamoxifen is planned in a very high risk population identified by traditional risk factors to determine whether marker expression changes with tamoxifen administration. Serial specimens would be examined for modulation of marker expression in response to the intervention. Pharmacologic investigations are proposed to examine the lowest dose of these agents which are associated with a biologic effect.

In addition, women with abnormalities on screening mammography would be stratified by whether biopsy of the abnormality was recommended based on standard clinical practice. The characterization of the association between mammographic findings and biomarker expression should be a useful adjunct to the management of these women.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00181-01 BPRB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Molecular Genetics of Gynecologic Cancers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. J. Birrer	Senior Investigator	BPRB, EDCOP, DCPC, NCI
Others:	S. Lemon	Cancer Prevention Fellow	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS *(if any)*
Department of OB-GYN, Navy Medical Center (M. Teneriello, R. Taylor, J. Nash)
Armed Forces Institute of Pathology (J. Norris)

LAB/BRANCH
Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Gynecologic cancers remain a major problem in this country with approximately 25,000 deaths annually attributed to these diseases. The purpose of this project is to characterize the molecular genetics of this group of tumors and ultimately use that information for clinical applications in designing therapeutic and prevention trials

We have characterized a group of ovarian tumors which span the histologic spectrum from benign cystadenomas through tumors of "low malignant potential" (LMP) to ovarian carcinomas for mutations in the *ras* and p53 oncogenes. Results from this study revealed that while benign and LMP tumors possessed activated *ras* genes, ovarian carcinomas did not. In addition, mutations in p53 were frequent in ovarian carcinomas but not in LMP tumors. This suggests that these tumors are discrete biologic entities.

We are also determining the molecular "signature" of uterine sarcomas and leiomyomas, and various endometrial specimens which span the histologic spectrum from benign to malignant. These studies will help to identify the molecular genetic events which are important in the genesis of these tumors. In addition, it will characterize the temporal relationship among these events enabling one to determine if any of these lesions can be used as markers of early disease.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00182-01 BPRB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemistry of Peptides and Growth Factors in Lung Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.W. Moody	Section Head	BPRB, EDCOP, DCPC, NCI
Others:	S. Jakowlew	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	A. Mathias	Technician	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS *(if any)*
George Washington University Medical Center, Washington, DC (F. Zia)
University of Arizona College of Medicine (T. P. Davis)
Tel Aviv University (I. Gozes)

LAB/BRANCH
Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION
Experimental Biochemistry Section

INSTITUTE AND LOCATION
National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	1.5	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The biochemistry of peptides and growth factors in lung cancer was investigated. High affinity neuromedin B (NMB) binding sites were detected in small cell lung cancer (SCLC) cells. Because NMB elevated the cytosolic Ca+*2* in Fura-2 AM loaded NCI-H345 cells, NMB similar to gastrin realeasing peptide (GRP) may stimulate phosphatidylinositol (PI) turnover. GRP receptor antagonists such as (D-FPhe*6*, D-Ala*11*)BN*6-13* methyl ester ((FA)BN*6-13*ME) only weakly antagonized the ability of NMB to elevate cytosolic Ca+*2* or stimulate clonal growth. These data suggest that SCLC cells have distinct GRP and NMB receptors. Also, the GRP receptor was solubilized and purified using affinity chromatography techniques. A major 65 Kdalton band was isolated and the GRP receptor is a G-protein coupled receptor containing 384 amino acid residues and 7 hydrophobic domains.

Vasoactive intestinal peptide (VIP) mRNA was detected in high concentrations in NCI-H727 cells. Also, immunoreactive VIP was detected in lung cancer cell extracts and conditioned media. Pituitary adenylate cyclase activating peptide (PACAP) and VIPhybrid inhibited *125*I-VIP binding with high and moderate affinity respectively. VIP and PACAP elevated intracellular cAMP levels whereas VIPhybrid inhibited the increase in cAMP caused by VIP. Also, VIP and PACAP stimulated lung cancer clonal growth whereas VIPhybrid inhibited colony formation. VIP hybrid inhibited lung cancer xenograft formation in nude mice. These data indicate that VIPhybrid is a lung cancer VIP receptor antagonist. Also, specific *125*I-PACAP binding was inhibited with high affinity by PACAP but moderate affinity by VIP. PACAP elevated the cytosolic Ca+*2* in addition to elevating intracellular cAMP. These data suggest that PACAP binds to a unique receptor in addition to binding with high affinity to VIP receptors.

GRP receptor antagonists may be useful for the treatment of SCLC. VIP receptor antagonists such as VIPhybrid have broader applicability in that they inhibit proliferation of both SCLC and NSCLC cells.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CN 00183-01 BPRB

PERIOD COVERED
June 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of Markers for the Early Detection of Lung Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. L. Shaw	Senior Investigator	BPRB, EDCOP, DCPC, NCI
Others:	J. L. Mulshine	Chief	BPRB, EDCOP, DCPC, NCI
	F. Cuttitta	Deputy Chief	BPRB, EDCOP, DCPC, NCI
	S. Jakolew	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	E. Szabo	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	H. Pass	Head	TOS, SB, COP, DCT, NCI

COOPERATING UNITS *(if any)*
Los Alamos Cancer Project, Los Alamos National Laboratory (D. Cole)

LAB/BRANCH
Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.75	1.25	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The early detection of lung cancer is critical to improving the mortality rate associated with lung cancer. Studies from the Lung Cancer Study Group have shown that the 5-year survival of patients with very early stage non-small cell lung cancer (T1N0M0) is 80%, far better than the overall 10% 5-year survival for lung cancer. We have had an ongoing collaboration with Dean Cole at the Los Alamos National Laboratory to evaluate a new photoactive porphyrin compound for the detection of early lung neoplasms, and use a radiolabeled form of this compound for local ablation of abnormal bronchial epithelium (in project Z01 CN 00169-02 BPRB). This project is awaiting further development of the porphyrin compound.

During Summer 1993, protocols for the early detection of lung cancer among individuals at high risk will be submitted for approval. We propose to target lung cancer and head and neck cancer survivors with serial monitoring of sputum and bronchoscopically obtained specimens to assay for biomarker expression. The study design will incorporate comparison of findings in different specimens such as bronchial washings, bronchial biopsies at multiple sites and expectorated sputum. Particularly important would be whether or not the markers are differentially expressed in the unaffected portions of the lung or only detectable in certain types of specimens.

A subset of subjects will be enrolled in an intervention trial. Specimens will be obtained at on-study, after the period of intervention and roughly 3 months after the intervention is stopped. The first intervention trial anticipated will use 4-HPR if available, or low dose 13-*cis*-retinoic acid. Future intervention agents for this subject population might include Vitamin E or the radiolabeled photoactive porphyrin compound for a 3-6 month period. Pharmacokinetic studies will also be incorporated to evaluate the effect of dose on marker modulation as well as to evaluate alternative forms of administration such as aerosolization of the agents. The success of early detection of cancer is dependent upon the ability to intervene successfully at that early stage to prevent the morbidity and mortality associated with cancer and standard therapy.

Published reports have suggested that genomic p53 mutations may be present in individuals with an inherited susceptibility to several types of cancer, including but not limited to Li-Fraumeni syndrome. A similar study using genomic DNA from whole blood collected during a case-control study of lung cancer is planned to evaluate markers of susceptibility to lung cancer.